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T-cell homing specificity and plasticity: new concepts and future challenges

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Naive and effector/memory T cells have distinct repertoires of trafficking ligands and receptors that restrict their ability to interact with specialized microvessels in different anatomical compartments and, consequently, have distinct patterns of migration. Antigen-experienced lymphocytes can be further subdivided into different subsets based on their expression of characteristic sets of trafficking receptors that favor their accumulation in certain target organs, including the skin and gut. Here, we summarize recent advances that have broadened our understanding of the cellular and molecular events that induce the generation of tissue-specific effector/memory T cells and discuss how these mechanisms could be harnessed for the therapeutic manipulation of T-cell-dependent pathologies.

Introduction

To perform their immunological functions, T cells must exit the blood and enter into different tissues in the body. An essential step in this process is their adhesion to the endothelium of postcapillary venules, a complex, multistep cascade of events mediated by various adhesion receptors [1]. Although the multi-step paradigm applies to all leukocytes, the molecules involved in the different steps vary depending on the leukocyte population, the target tissue and the inflammatory context [1] (Table S1 in the online supplementary material). Recent advances in the field have unveiled several examples of this exquisite degree of specialization, in particular, for T-cell migration. Indeed, many T-cell subsets express unique patterns of homing molecules to interact with organ-specific microvascular endothelial cells for preferential recruitment to distinct target tissues. Recent observations highlight a previously unexpected degree of dynamic plasticity in the trafficking behavior of effector/memory T cells. These findings open the possibility that the expression of tissue-specific molecules on lymphocytes could be reprogrammed and custom-modified to tailor cellular immune responses for therapeutic purposes.

Migration of effector/memory T cells: what can T cells remember from their past experiences?

Naive T cells preferentially migrate to secondary lymphoid organs (SLO), including peripheral or mesenteric lymph nodes (PLN or MLN), Peyer's patches (PP) and the spleen, which are the sites where T cells first meet their cognate antigen and become activated if they encounter appropriate co-stimulatory signals. Antigen-experienced T cells are more diverse than naive T cells with respect to their migratory properties. In particular, effector/memory cells migrate to a greater extent to non-lymphoid tissues and sites of inflammation [2,3], and are subdivided into several categories based on distinct migratory and functional characteristics [4] (Figure 1; Table S1 in the online supplementary material). Although there are notable differences between $CD4^+$ and $CD8^+$ T cells with respect to the molecular signals that induce and maintain different effector/memory populations, the antigen-experienced cells in both categories have been divided into central memory (T_{CM}) and effector memory (T_{EM}) subsets [4]. T_{CM} express the LN homing receptors L-selectin and CCR7 and, like naive T cells, are well represented in all SLO [3]. They also express inflammation-seeking trafficking molecules and high levels of the chemokine receptor CXCR4, which enables them to migrate efficiently to sites where the ligand for CXCR4, the chemokine CXCL12, is expressed, including the bone marrow [5] and PLN [6]. Consistent with their memory status, T_{CM} express the interleukin receptor IL-7Rα [7] and respond faster and more vigorously than naive T cells when re-encountering cognate antigen [4,8,9].

In contrast to T_{CM}, effector T cells (T_{EFF}, which are short-lived) and T_{EM} (which are long-lived and IL-$7R\alpha^+$) do not express CCR7, and most are also L-$selectin^{-/Low}$. Therefore, T_{EM} and T_{EFF} cannot recirculate efficiently through LN or PP [3,4], but they do migrate to peripheral and non-lymphoid tissues [2–4]. In addition, in some assays, only T_{EFF} and T_{EM}, but not T_{CM}, have immediate effector cytokine producing and/or cytotoxic activity [4,8]. However, it should be cautioned that in some studies *in vivo* differentiated T_{CM} function as effectors as rapidly and efficiently as T_{EM} [10,11], and T_{CM} that arise following viral infections are better than T_{EM} at conferring long-lived antiviral protection [9]. Moreover, the migratory routes of $CCR7^+$ memory cells are not restricted to SLO and, depending on precursor frequency, T_{EM} have been observed to convert back to a T_{CM} phenotype [9,12]. Thus, the distinctions between T_{CM} and T_{EM} can be rather subtle. Here, we use 'T_{CM}' to refer to antigen-experienced T cells with PLN-homing capacity (i.e. L-$selectin^{High}$ and $CCR7^+$), and 'T_{EM}' and 'T_{EFF}' to refer to antigen-experienced T cells that lack one or both PLN homing receptors. We can distinguish between T_{EM} and T_{EFF}

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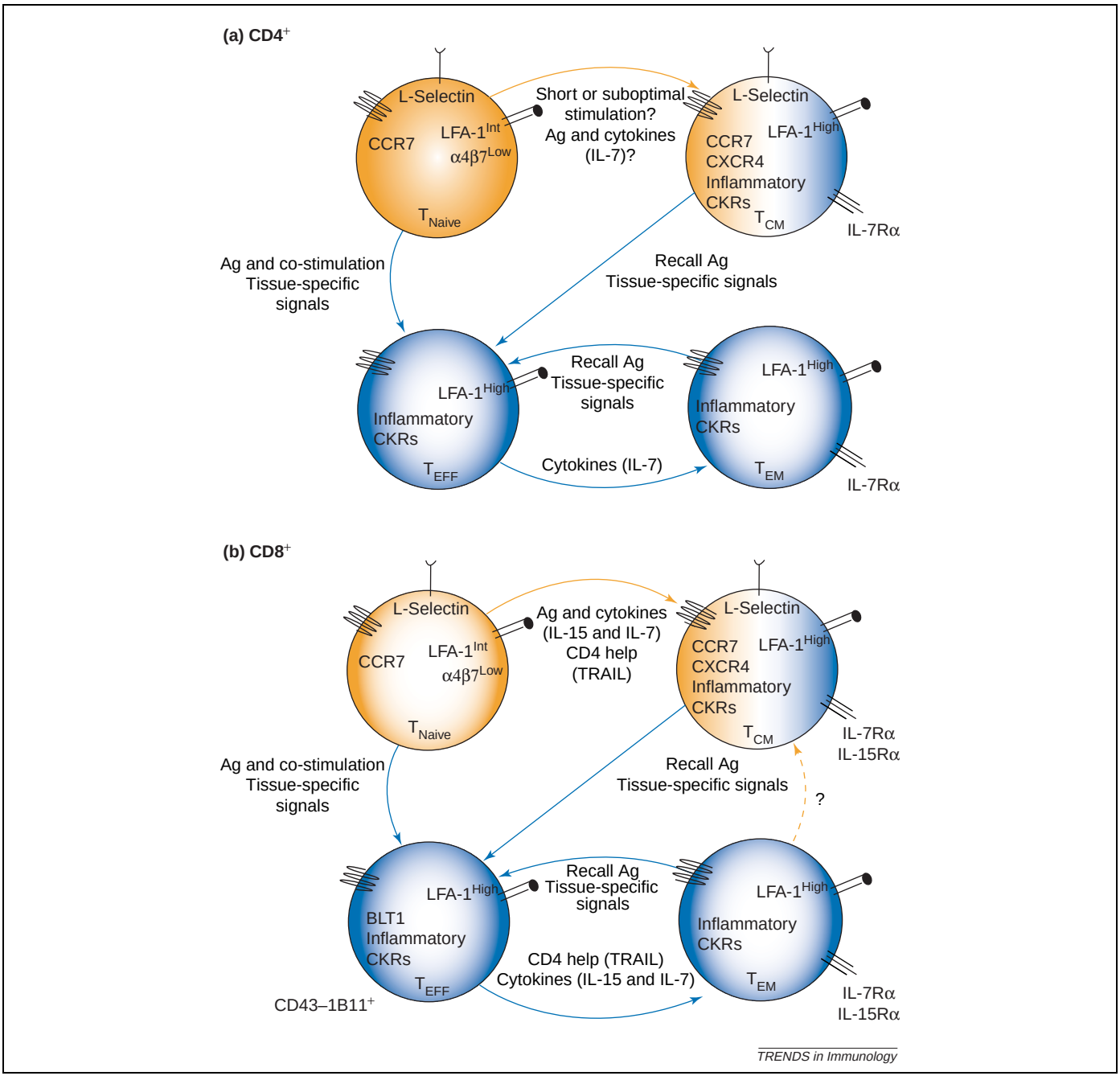

Figure 1. Differentiation and migratory pathways of naive and antigen-experienced T cells. Naive $CD4^+$ **(a)** and $CD8^+$ **(b)** T cells (T_{naive}) express L-selectin, CCR7, intermediate (Int) levels of LFA-1 and low levels of α4β7, which enable them to migrate into SLO, such as LN, PP and the spleen (orange indicates an SLO-homing phenotype). Following activation by their cognate antigen (Ag), T cells differentiate into T_{CM} or T_{EFF}; some of the T_{EFF} can give rise to T_{EM}. T_{CM} and T_{EM} are long-lived because they express receptors for IL-7 ($CD4^+$ and $CD8^+$ T cells) and IL-15 ($CD8^+$ T cells), which provide essential signals for survival and homeostatic proliferation. T_{CM} remain L-selectin$^+$ and $CCR7^+$ and continue to recirculate through SLO. In addition, T_{CM} can migrate to inflamed tissues and the bone marrow, and they respond more vigorously to antigen than do T_{Naive}. The short-lived T_{EFF} and long-lived T_{EM} also respond efficiently to recall (re-encountered) antigen and exert rapid effector activity, such as secretion of effector cytokines (Th cells) or cytotoxicity (cytotoxic T lymphocytes). T_{CM}, T_{EFF} and T_{EM} express trafficking molecules that confer the ability to migrate to non-lymphoid tissues and sites of inflammation (blue). T_{CM} and T_{EM} give rise to T_{EFF} upon reactivation. $CD8^+$ T_{EM} might also give rise to T_{CM}, at least in some experimental settings; the extent to which this occurs physiologically is unclear. Abbreviations: BLT1, leukotriene B4 receptor; IL-7Rα and IL-15Rα, α subunits of the IL-7 and IL-15 receptors.

because T_{EM} are long-lived whereas T_{EFF} represent mostly short-lived recently activated T cells.

Tissue-specific homing receptors: distinct 'zip codes' to shape immune responses

T_{EFF} and T_{EM} share, by definition, the inability to migrate to sites that require CCR7 and L-selectin expression (i.e. SLO other than the spleen), but they are by no means homogeneous with respect to their preferred tissue targets. Major T_{EFF} and T_{EM} subsets have been identified that show remarkable migratory selectivity for certain organs, including the gut and skin (Figure 2a; Table S1 in the online supplementary material). Murine cutaneous T_{EFF} and T_{EM} express E- and P-selectin ligands [13] and the chemokine receptors CCR4 [14] and/or CCR10 [15], which are crucial for efficient T-cell homing into the skin [16–18]. E- and P-selectin are upregulated on endothelial cells exposed to inflammatory mediators in many tissues.

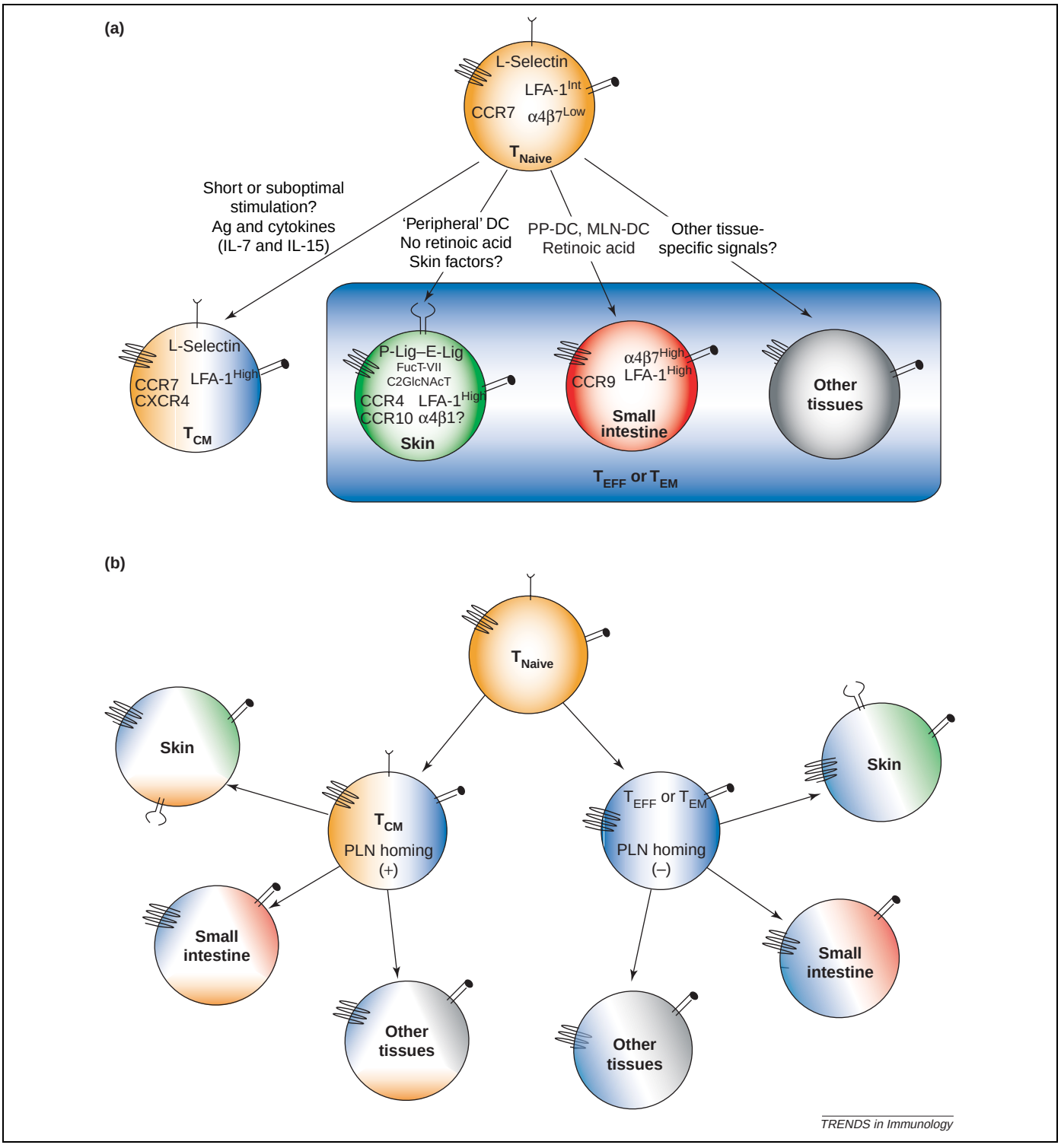

Figure 2. Two models of T-cell differentiation and tissue-specific imprinting. **(a)** In the conventional model, naive T cells are activated by a cognate antigen, and depending on the strength and/or 'quality' of the stimulation and/or on the presence of some cytokines (e.g. IL-7 and IL-15), they differentiate into either T_{CM} or T_{EFF}/T_{EM}. In addition, if T cells are activated by PP-DCs or MLN-DCs or in the presence of RA, the resulting T_{EFF}/T_{EM} become $\alpha4\beta7^{High}$ and $CCR9^{+}$. These cells migrate particularly efficiently to the small intestine. By contrast, when T cells are activated in the absence of RA or by 'peripheral-DCs' (e.g. PLN-DCs), they upregulate ligands for P-selectin (P-Lig) and E-selectin (E-Lig) and receptors for skin-expressed chemokines, which allow them to home to the skin. There are probably additional mechanisms for T-cell targeting to other organs (including the CNS, lungs and synovium) but these are not shown for clarity. These mechanisms have been mostly characterized in mice and under non-inflamed 'steady-state' conditions. Although there is an overlap, it is not known to what extent these mechanisms are homologous in humans and whether they also fulfill an essential role under inflammatory conditions. Colors indicate migratory preference: orange, SLO; blue, inflamed tissue; green, skin; red, small intestine; gray, other sites. **(b)** Alternatively, naive T cells might be exposed to several differentiation signals that independently (and not necessarily sequentially) shape their migratory phenotype. Factors such as the strength of the antigenic signal and associated cytokines (such as IL-2) could determine whether or not memory cells lose the capacity to home to LN. Tissue-specific imprinting signals provided by DCs (e.g. in the form of RA), independently control the peripheral tissue preferences of the T cell. Additional signals, such as the effector cytokine milieu (i.e. Th1/2 cytokines) might modulate imprinting (not shown). The final trafficking properties of each T cell will be determined by the combination of responses to diverse differentiation signals that each trigger distinct migratory subroutines. FucT-VII: fucosyltransferase-VII; C2GlcNAcT: Core 2 1,6-*N*-acetylglucosaminyltransferase. Colors are as in (a).

However, unlike most other microvascular beds, skin venules express functional E- and P-selectin constitutively, as shown by the high frequency of rolling leukocytes in non-inflamed skin [19]. CCR4 and/or CCR10 have a role in cutaneous T-cell homing under steady-state and inflammatory conditions [17,18], although other chemokine receptors, such as CXCR3, contribute in acute inflammatory states [20]. The ligands for CCR4 and CCR10 (CCL17 and CCL27, respectively) have been found on inflamed and non-inflamed skin endothelium [14,18]. In human skin, most resident T cells express CCR8, but the functions of CCR8 and its skin-expressed ligand CCL1 remain to be determined [21].

Gut-tropic T cells migrate preferentially to the lamina propria of the small intestine, and they do not have skin homing receptors but express high levels of the integrin $\alpha4\beta7$ [22,23] and the chemokine receptor CCR9 [24]. These trafficking molecules are essential for efficient T-cell migration into the small bowel, at least in the absence of inflammation [23,25–28]. Accordingly, the principal $\alpha4\beta7$ ligand, MAdCAM-1, is expressed in gut lamina propria venules [29], and the CCR9 ligand CCL25 is strongly expressed by epithelial cells in the small intestine and in lamina propria venules [28,30]. Genetic ablation of CCR9 causes only a mild reduction in a subset of small bowel intraepithelial lymphocytes that expresses the T-cell receptor (TCR) γ and δ subunits, suggesting that other, as yet unknown, chemoattractants can maintain intestinal lymphocyte homeostasis [31]. Nevertheless, CCL25 blockade or CCR9 deficiency significantly reduces the number of antigen-specific $CD8^+$ T cells in the small intestine following intraperitoneal immunization [26,27]. It is possible that mice with inborn CCR9 deficiency develop compensatory mechanisms to recruit T cells to the gut. Alternatively, CCR9 might be more important for the antigen-induced migration of recently generated $CD8^+$ T_{EFF} to the small bowel, rather than for steady-state traffic, which has a slow turnover [32]. A substantial proportion of $CD4^+$ T_{EFF} migrate to the small intestine in a CCR9-independent fashion, suggesting an alternative pathway of T-cell migration into this compartment [33].

Interestingly, T-cell homing into the large intestine (colon) is controlled, at least in part, by mechanisms distinct from those operating in the small intestine. Although integrin $\alpha4\beta7$ is important for T-cell migration into this gut compartment, even under inflammatory conditions [34], integrin $\alpha4\beta1$ has also been implicated [35]. The colon is mostly devoid of CCR9-expressing cells, and CCL25 is not expressed in this tissue [24,30]. Accordingly, CCR9 desensitization (or CCL25 blockade) significantly blocks the adhesion of T cells in small bowel venules but has no effect on T-cell adhesion in the colon [28]. These results suggest that there could be other chemoattractant pathway(s) that direct T-cell migration to the colon mucosa.

A widely held view is that peripheral tissue tropism is the sole domain of T_{EFF} and T_{EM}. This predicts that primed T cells are given a single set of mutually exclusive choices; they can either become T_{CM}, and keep recirculating through SLO, or they can lose LN homing receptors, and acquire peripheral tissue tropism (Figure 2a). However, although the $CCR7^+$ L-$selectin^{High}$ phenotype predicts the capacity of a memory cell to home to PLN [3,36], this does not preclude T_{CM} from infiltrating peripheral tissues [37] and sites of inflammation [3]. CCR7 has been detected on T cells in diverse peripheral tissues [38,39], where CCR7 could allow cells resident in the tissue to migrate into CCR7 ligand-expressing draining lymphatics and enter downstream LN via the lymph [38,39]. This suggests a more complex scenario, whereby primed T cells make (at least) two decisions: whether to retain or discard CCR7 and L-selectin and, independently, whether to acquire organ-specific peripheral homing receptors (Figure 2b).

Subsets of $CD4^+$ $CD25^+$ ($Foxp3^+$) regulatory T cells (T_{REG}) also show tissue specificity, particularly T_{CM}-like and T_{EM}-like phenotypes, with T_{CM}-like T_{REG} exerting their regulatory activity in SLO and T_{EFF}-like T_{REG} acting in peripheral inflamed tissues [40]. It will be interesting to explore whether tissue specificity (e.g. gut- versus skin-tropic T_{REG}) is also induced during T_{REG} activation. In fact, a recent report suggests that T_{EFF}-like T_{REG} need fucosyltransferase-VII (FucT-VII), an enzyme that is essential for the biosynthesis of P- and E-selectin ligands, in order to exert their regulatory activity in the skin [41].

How effector/memory T cells learn where to go – the instructive role of dendritic cells

In humans, the site of antigen entry strongly influences the traffic pattern of T_{EFF}. Pathogens entering through the skin, for example, herpes simplex virus, preferentially prime lymphocytes with skin homing receptors [42–44]; by contrast, oral vaccination induces higher levels of $\alpha4\beta7$ on effector/memory T cells (suggesting homing to the gut) than intramuscular or subcutaneous administration of the same antigen [45–47]. Importantly, among memory $CD8^+$ T cells, only $\alpha4\beta7^+$ (but not $\alpha4\beta7^-$) cells carry protection against intestinal rotavirus infection following adoptive transfer by intravenous transfusion to naive mice [48].

In agreement with these observations, the homing potential of activated lymphocytes depends on the lymphoid tissue environment; T cells activated in MLN express higher levels of $\alpha4\beta7$ and CCR9 than do those activated in skin-draining PLN [26,49]. Conversely, skin homing receptors (selectin ligands) are preferentially induced when T cells are activated in dermal PLN [49]. A recent study found that $CD8^+$ T cells primed in cervical LN and receiving antigens derived from the central nervous system (CNS) acquire high levels of $\alpha4\beta1$ and P-selectin ligands. These changes confer the capacity on T_{EFF} to migrate to the brain [50]. Interestingly, when two different TCR-transgenic $CD8^+$ T cells were simultaneously stimulated in the same PLN with tumors administered intraperitoneally or through the skin, they acquired high levels of integrin $\alpha4$ or P-selectin ligands, respectively, suggesting that the anatomical source of the antigen-presenting cells is dominant over environmental factors in the draining LN [50].

Dendritic cells (DCs) are essential for efficient T-cell priming. They influence many effector functions of T and B cells, including bias towards T helper 1 (Th1) or Th2 status and antibody isotype switching, often in a tissue-specific

fashion [51]. DCs are also responsible for the imprinting of tissue-specific homing potential. T-cell activation by intestinal DCs from PP (PP-DCs) and MLN (MLN-DCs) induces gut-homing capacity [27,52–55] (Figure 3). By contrast, DCs from PLN (PLN-DCs) induce higher levels of selectin ligands than intestinal DCs [54–56]. Consistent with the finding that FucT-VII is essential for selectin ligand generation [57], PLN-DCs induce higher levels of FucT-VII mRNA in $CD8^+$ T cells than do PP-DCs [54]. Interestingly, $CD8^+$ T-cell stimulation with splenic DCs or glutaraldehyde-fixed DCs from any SLO or with anti-CD3 and anti-CD28 antibodies (without DCs) always induces selectin-ligand^{High} T_{EFF} [54]. Thus, the acquisition of skin homing molecules, at least selectin ligands and probably also CCR4, could be a default response whenever T cells are activated in the absence of gut-derived imprinting signals [54]. Indeed, TCR stimulation and activation of the Ras–MAP kinase pathway in T cells are sufficient to upregulate FucT-VII and P- and E-selectin ligands [58,59].

CCR4 can also be induced on activated T cells *in vivo* by immunization routes other than the skin [56], suggesting that the upregulation of this chemokine receptor does not require skin-specific signals. However, TCR stimulation alone does not induce CCR10 or CCR8 (which is expressed on human skin-resident T cells [21]), implying that these chemokine receptors could be controlled by skin-specific cues. By contrast, CCR10 is also expressed in IgA-secreting cells [60], and CCR8 is upregulated in Th2 cells [61]. Thus, the mechanisms regulating the expression of these receptors are complex.

DC-derived Th1 differentiation signals, in particular IL-12, can efficiently induce P- and E-selectin ligands on T

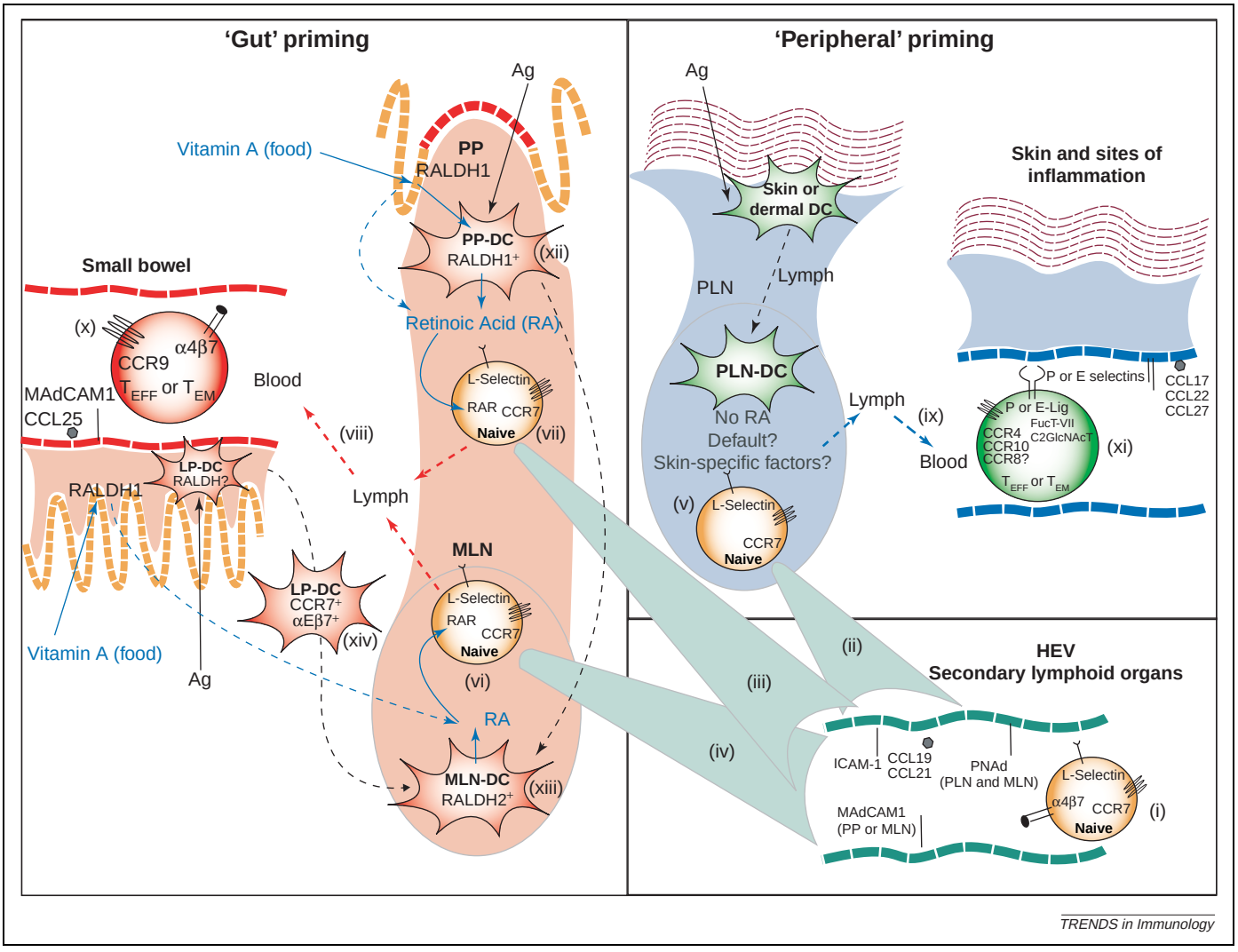

Figure 3. Imprinting mechanisms for gut- and skin-specific T cells. Naive T cells (i) access PLN (ii), PP (iii) and MLN (iv) through high endothelial venules (HEV) expressing essential vascular addressins [2]. Homed cells become activated in the T cell area of SLO (v–vii) where they encounter a cognate antigen on mature DCs. Once a lymphocyte has become stimulated, it gives rise to tissue-seeking T_{EFF} that leave the SLO via efferent lymphatics and enter the blood (viii,ix) [T_{EFF} from the spleen (not shown) enter the blood directly] and home to peripheral organs through specialized postcapillary venules that express tissue-specific trafficking molecules (x, xi). PP-DCs (xii) and MLN-DCs (xiii) express crucial enzymes for metabolizing vitamin A (retinol) into RA, particularly RALDH-1 and -2. The encounter between T cell and antigen in the presence of DC-derived RA induces gut homing receptors and suppresses skin homing molecules. This requires RA signaling through the RAR family of RA-receptors. Mature $\alpha E\beta 7^+$ DCs from the lamina propria [LP-DC, (xiv)] migrate to the draining MLN, where they probably serve as a source of RA. In addition, RA might be synthesized by other cells in the intestinal mucosa, such as enterocytes, which express RALDH-1. PP-DCs (xii) could also migrate into MLNs and contribute to the pool of MLN-DCs (xiii). When T cells are activated in skin-draining PLN (v), they are exposed to DCs that cannot synthesize RA. The T cells then upregulate skin homing molecules. The induction of selectin ligands and probably also CCR4 (xi) seems to reflect the default differentiation pathway that T cells take upon stimulation without other tissue imprinting signals. However, it is still possible that skin-associated DCs or other environmental factors are needed to upregulate CCR8 or CCR10 on cutaneous T cells.

cells stimulated *ex vivo*, whereas the Th2 cytokine IL-4 suppresses their expression [62]. However, this correlation is not observed with Th1 and Th2 cells differentiated *in vivo* [63,64]. In fact, blocking IL-12 and/or interferon (IFN)-γ does not abrogate the expression of selectin ligands on $CD8^+$ T cells activated by DCs from PLN, although IL-12 addition enhances their expression [54]. Consistent with this, FucT-VII can be upregulated in a manner independent of IL-12 or STAT4 signaling, which are essential for Th1 differentiation [58], and IL-12 is not necessary for the induction of P-selectin ligands on $CD8^+$ T cells activated *in vivo* [65]. A recent report showed that T cells deficient in T-bet (a transcription factor essential for Th1 differentiation) and differentiated *ex vivo* under conditions that produce Th1 cells, are impaired in their ability to upregulate P-selectin ligands [66]. It will be important to determine whether T-bet is similarly required under *in vivo* priming conditions. Thus, although a lymphoid milieu rich in effector cytokines might modulate the expression of trafficking molecules on T_{EFF}, such as Th1- or Th2-associated chemokine receptors, the generation of tissue tropism does not seem to be controlled by Th1 or Th2 commitment.

Imprinting mechanisms for gut-homing T cells

The imprinting mechanism for gut-homing T cells was uncovered by Iwata *et al.* [67], who analyzed vitamin A-deficient mice. These animals had dramatically reduced numbers of effector/memory T cells in the gut mucosa, but the T-cell compartment remained unaffected elsewhere [67]. Experiments i*n vitro* showed that the presence of the vitamin A metabolite retinoic acid (RA) during T-cell activation induces α4β7 and CCR9 and reduces the upregulation of CCR4 mRNA, even in the absence of DCs. RA also suppresses the activation-induced default upregulation of FucT-VII and E- and P-selectin ligands [67]. The same skewed differentiation is observed when T cells are activated in the presence of PP-DCs [54].

These two observations are causally linked; DCs from PP and MLN, unlike those from spleen or PLN, express high levels of retinaldehyde dehydrogenases (RALDHs), which are essential enzymes during the biosynthesis of RA from vitamin A [67]. Blocking RALDHs in DCs or RA receptors in T cells significantly decreases the induction of α4β7 by PP-DCs and MLN-DCs [67]. The ability to produce RA is therefore an important mechanism by which intestinal DCs induce the gut-homing properties and suppress the skin-homing properties of T cells (Figure 3). A recent study has identified a subpopulation of MLN-DC that express integrin αEβ7 (also called CD103) and are particularly potent at inducing CCR9 on activated T cells [68]. It will be interesting to determine RALDH expression and activity in these $\alpha E\beta7^+$ MLN-DCs and how or why the expression of αEβ7 is correlated with preferential gut imprinting capacity.

It should be emphasized that, although PP-DCs generate T cells that migrate to the small intestine, PP-DC-induced T_{EFF} home poorly to the colon [53], suggesting that additional unknown factors could be involved in the imprinting of colon-homing T cells (assuming that specific colon-tropism exists). In addition, although it is apparent that T cells can be educated by DC-derived RA to acquire gut-homing potential *in vitro* [27,53–55,67], additional mechanisms might operate *in vivo* to control T-cell traffic. For example, differences in proliferation and/or survival might account for the preference of some lymphoid populations to accumulate in particular tissues, such as MLN [69]. However, preferential T-cell migration into the gut or to the inflamed skin can be observed after short-term adoptive transfer, even when cognate antigen is not present in the host [53,54]. Thus, peripheral tissue tropism is not merely an effect of T-cell selection at a target site, although the presence of cognate antigen can boost the accumulation of lymphocytes in some peripheral organs [70].

Most studies on the location of effector/memory T cells in non-lymphoid tissues have focused on entry (homing) or proliferation and survival as determining factors of lymphocyte content in a given tissue. However, two recent studies indicate that the rate of exit through the draining lymphatics might also have a role. Control of T-cell exit by this route depends, at least partly, on CCR7 [38,39]. It will be important to determine whether there are other chemoattractant pathways involved in this process and whether there are tissue-specific differences. $CD8^+$ T cells upregulate αEβ7 and the activation marker CD69 following homing into the small bowel mucosa [32,71]. Given that αEβ7 is important for interactions between T cells and intestinal epithelial cells [71] and CD69 has been implicated in the retention of T-cell precursors in the thymus [72], it is tempting to speculate that these two molecules might also control T-cell exit and/or survival in the gut mucosa.

It has been suggested that T cells could also acquire homing receptors stochastically, and that the peripheral tissues with their traffic molecules, known as vascular addressins, would select the 'correct' T cells [73]. A recent study showed that localized viral infections give rise to effector/memory $CD8^+$ T cells that can locate to many non-lymphoid tissues, including the small bowel [74]. Although it is not unexpected that effector T cells can localize to peripheral tissues such as the lungs and the liver [3], it is interesting to note that infection with Sendai virus (which is mostly restricted to the respiratory mucosa) also generates antigen-experienced $CD8^+$ T cells that localize to the gut mucosa [74]. It is possible that virus antigens have been carried away and presented in gut-associated lymphoid tissues in this setting.

Although intestinal DCs are sufficient to induce gut-homing molecules *in vitro*, other sources of RA could potentially contribute *in vivo*. For example, enterocytes in the small intestine express RALDH-1 [67] (Figure 3), and intraperitoneal immunization efficiently generates gut-homing T cells [26,27,49]. Thus, it is possible that gut-homing T cells can be imprinted in regions other than gut-associated lymphoid tissues. It will be interesting to determine whether RA synthesis can be induced in non-intestinal DC under inflammatory or infectious conditions and whether there are additional, RA-independent mechanisms of intestinal imprinting [75]. Moreover, the expression of α4β7 and CCR9 is not always linked. For example, naive $CD8^+$ T cells express high levels of CCR9

[76] but low levels of α4β7 [53], and T cells infiltrating the colon mucosa are $\alpha4\beta7^{High}$ but $CCR9^{-}$ [24]. As RA induces both these gut homing molecules simultaneously [67], these findings suggest that there are additional signals that can regulate individual gut homing molecules separately [75].

Plasticity in T-cell homing: reprogramming T cells to reach new horizons

There is mounting evidence that the tissue commitment of effector/memory lymphocytes is often reversible. For example, human $CD4^{+}$ T cells can sequentially up- or downregulate the skin homing receptor CLA (an E-selectin ligand) when they are reactivated under Th1 or Th2 conditions, respectively [63]. In addition, effector/memory $CD8^{+}$ T cells isolated from intestinal mucosa were inefficient at homing back to the gut [74]. Indeed, although α4β7 is needed for efficient T-cell homing to the intestine, most $TCR\alpha\beta^{+}CD8\alpha\beta^{+}$ T cells in the gut are $\alpha4\beta7^{Low/-}$ [53], presumably because α4β7 is downregulated and αEβ7 is upregulated soon after T cells reach the intestinal mucosa [71]. Conversely, reactivation of initially non-gut-tropic $CD8^{+}$ T_{EM} with a systemic viral challenge induces the appearance of virus-specific cells in the gut [74], suggesting that some effector/memory T cells can be 're-educated' to acquire gut-homing properties. In fact, skin- and gut-homing commitment are not immutable, but apparently reflect dynamic functional states. *In vitro* studies show that T cells that have been imprinted with gut- or skin-homing potential by intestinal DCs or PLN-DCs, respectively, can be readily 'reprogrammed' when they are re-stimulated by DCs from a different SLO [54,55] (Figure 4).

Re-education of memory lymphocytes might also be possible in humans. When volunteers were immunized orally or parenterally and then re-immunized with *Salmonella* through the same or the opposite route, the last immunization was dominant over the first, at least with respect to the induction of α4β7 on antigen-specific antibody-secreting B cells [77]. Thus, memory B cells also appear to possess plasticity in their homing commitment. Tissue selectivity seems to be a dynamic and pliable property of memory lymphocytes that could be exploited for therapeutic intervention. For example, the pharmacological provision of homing instructions could enable improved targeting of effector/memory lymphocytes to improve vaccination protocols in infectious diseases or cancer. Alternatively, it might be possible to divert pathogenic lymphocytes away from a site of autoimmune attack to other tissues where the redirected cells would remain harmless.

Future directions

A growing body of evidence indicates that the division between T_{CM} and T_{EFF}/T_{EM} can be complex and fluid, at least with respect to immunological function. Aside from the presence or absence of CCR7 and L-selectin, respectively, additional universally accepted criteria for unambiguous distinction between these T cell subsets remain lacking. *In vitro*, T_{CM}-like cells can be produced from antigen-primed naive $CD4^{+}$ precursors by exposing them

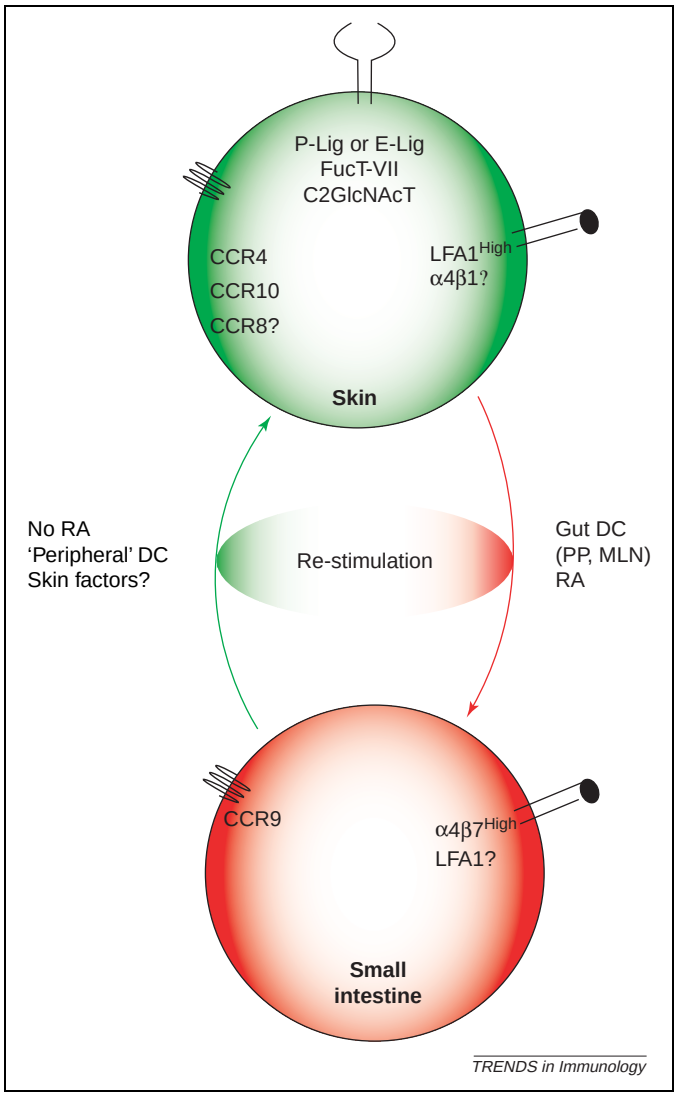

Figure 4. Plasticity in lymphocyte homing potential. Effector/memory T cells with gut-homing potential (bottom) are rapidly converted to skin-homing T cells (top) if they are reactivated with peripheral DCs (i.e. in the absence of RA). Conversely, cutaneous effector/memory T cells readily acquire a gut-homing phenotype when they are re-stimulated by intestinal DCs or in the presence of RA.

to a short, non-polarizing antigenic stimulus [36], or from $CD8^{+}$ precursors by maintaining them in survival-promoting cytokines, for example, IL-7, IL-15 or low concentrations of IL-2 [8]. However, it is unclear how primed T cells decide *in vivo* whether to retain or lose LN homing receptors.

The molecular mechanisms driving the homing of effector/memory lymphocytes to the small intestine and the skin have been partially elucidated. Although gut DCs and RA seem to be necessary and sufficient to imprint T cells with small bowel tropism in the steady state, it cannot be ruled out that there are additional mechanisms to generate gut-homing lymphocytes under inflammatory conditions. In addition, T cells seem to require different signals to migrate into the large intestine [53], and it will be important to determine the specific multi-step cascade for homing to the colon and other putatively selective target organs, including the CNS, the joints and the lungs. In addition, it will be interesting to investigate whether, analogously to vitamin A in the gut, vitamin D3 (which is

produced in the skin) or its metabolites might contribute to the imprinting of skin-associated chemokine receptors, such as CCR10 and CCR8.

Given that gut-associated DCs have an active role in programming T cells to express gut homing molecules [67,75], it will be important to understand how DCs themselves are 'educated' to acquire this tissue-specific imprinting potential. It is likely that 'DC education' happens in the periphery after bone marrow-derived DC precursors have established a tissue residence, but the alternative, *a priori* specialization of precursors in the bone marrow, has not been ruled out. Wherever DC imprinting specialization has its origin, the signals involved in this process are unknown. Possible candidates include the gut microflora, signals through Toll-like receptors, the influence of epithelial and/or other stromal cells, intrinsic differentiation programs triggered during hematopoiesis, or a mixture of these factors.

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Supplementary data

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