The lymphoid system is equipped with a network of specialized platforms located at strategic sites, which grant strict immune-surveillance and efficient immune responses. The development of these peripheral secondary lymphoid organs (SLO) occurs mainly in utero, while tertiary lymphoid structures can form in adulthood generally in response to persistent infection and inflammation. Regardless of the lymphoid tissue and intrinsic cellular and molecular differences, it is now well established that the recruitment of fully functional lymphoid tissue inducer (LTI) cells to presumptive lymphoid organ sites, and their consequent close and reciprocal interaction with resident stroma cells, are central to SLO formation. In contrast, the nature of events that initially prime resident sessile stroma cells to recruit and retain LTI cells remains poorly understood. Recently, new findings revealed early phases of SLO development putting emphasis on mesenchymal and lymphoid tissue initiator cells. Herein we discuss the main tenets of enteric lymphoid organs genesis and focus in the most recent findings that open new perspectives to the understanding of the early phases of lymphoid morphogenesis.

Keywords: enteric lymphoid organ morphogenesis, stroma cells, LTI cells

INTRODUCTION
The lymphoid system possesses highly specialized peripheral organs formed at strategic anatomical sites that constitute three-dimensional platforms ensuring efficient immune-surveillance, rapid immune responses and maintenance of protective immunity. Secondary lymphoid organs (SLO), such as lymph nodes (LN) and Peyer’s patches (PP), develop during the embryonic life, but can also assemble after birth as it occurs with enteric cryptopatches and isolated lymphoid follicles (Randall et al., 2008; Eberl and Sawa, 2010; van de Pavert and Mebius, 2010; Nery et al., 2012). Remarkably, while LN develop at strictly invariant locations along lymphatic vessels, PP develop in variable number and position in the anti-mesenteric side of the mid-intestine (5–12 in mice; Nishikawa et al., 2003). Similarly, cryptopatches appear confined to intestinal lamina propria but they also distribute randomly within the gut wall (Kanamoto et al., 1996). Despite these intrinsic differences, SLO development relies on an antigen-independent process where presumptive regions are colonized by lymphoid tissue inducer (LTI) cells that cross-talk with resident mesenchymal cells through lymphotxin (LT) αβ, δβ2 and LTI receptor (LIR)βα3 interactions, thus creating a positive feed-back loop that culminates on the anlagen formation.

Although the mechanisms of SLO development have been extensively characterized throughout the years (Randall et al., 2008; van de Pavert and Mebius, 2010; Cupedo, 2011), most studies have been powerless to scrutinize early events preceding LTI cell colonization and clustering. Thus, putative early triggering events preceding LTI cell ingress into lymphoid organ anlagen remain poorly understood (Nishikawa et al., 2003).

GENESIS OF LYMPHOID ORGAN PRIMORDIA: THE LTI PARADIGM
Fetal hematopoietic cells colonize pre-defined sites between embryonic day 9.5 (E9.5) and 16.5 (E16.5) according to the type and location of the prospective lymphoid organ (Bennett et al., 1996; Adachi et al., 1998; Mebius et al., 2001; Yoshida et al., 2001; Veiga-Fernandes et al., 2007; Possum et al., 2011; Tachibana et al., 2011; Cherrier et al., 2012). Hematopoietic cells include CD3−CD4+−cKit+IL7Rα+Rorγt+/− LTI cells (Kelly and Scollay, 1992; Adachi et al., 1997, 1998; Mebius et al., 1997; Yoshida et al., 1999; Sawa et al., 2010; Possum et al., 2011; Cherrier et al., 2012) and a distinct population of CD3−CD4+−cKit+IL7Rα−CD11c+ lymphoid tissue initiator (LTI) cells (Hashi et al., 2001; Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007; Patel et al., 2012). PP development depends on LTI and ELIN cells, while LN genesis relies on LTI cells although the role of LTI in LN formation remains elusive. Upon arrival to prospective sites, LTI and LTI cells are believed to establish an interplay with their mesenchymal cell counterparts, lymphoid tissue organizers (LTO) cells, in order to trigger lymphoid organ formation.

The presence of fully functional LTI and ELIN cells is necessary for the development of enteric SLO. Absence of LTI cells, as described in mice deficient for Ikaros, Inhibitor of DNA-binding 2 (Id2), retinoic acid-related orphan receptor γt (Rorgt), and RUNT-related transcription factor 1 (Runx1) core-binding factor, beta 2 subunit (Cbfβ2), result in PP developmental failure (Wang et al., 1996; Yokota et al., 1999; Sun et al., 2006; Tachibana et al., 2011). Similarly, depletion of LTI cells or deficiency of Ret expression on these cells results in impaired PP formation (Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007).
Lymphoid tissue inducer cells express the chemokine receptors CXCR3 and CCR7 that specifically bind to the homoeostatic chemokines CXCL13 and CCL21/19, respectively. These chemokines create gradients that coordinate LTI cell migration and colonization of presumptive lymphoid organ sites (Forster et al., 1996, 1999; Honda et al., 2001; Luther et al., 2003; Mebius, 2003). In addition, the expression of the adhesion molecules ICAM-1, VCAM-1, and MadCAM-1 by stroma organizer cells ensures retention of hematopoietic cells through the ligation of the integrin receptors α4β1 and α4β7 expressed by LTI and LTin cells surface (Mebius et al., 1996; Hashi et al., 2001; Finke et al., 2002; Vega-Fernandez et al., 2007). Thus, it is commonly accepted that chemokines and adhesion molecules contribute to a productive and persistent communication between hematopoietic and mesenchymal cells (van de Pavert and Mebius, 2010).

The engagement of LTαβ2 expressed by LTI cells with stromal cell LTβR leads to activation of the classical and alternative NFκB signaling pathways, which are critical to stroma cell maturation and lymphoid organ development (Weih et al., 1995; Yamada et al., 2000; Aćamovic et al., 2001, 2002; Paxian et al., 2002; Yilmaz et al., 2005; Carragher et al., 2004; Lovas et al., 2008). In agreement, mice deficient for LTαβ2, LTβR, LTβR, or molecular players of the NFκB signaling pathways fail to develop LN and PP (Rennert et al., 1996, 1997, 1998).

The activation of LTβR results in the maturation of stroma cells, inducing the expression of adhesion molecules MadCAM-1, VCAM-1, and ICAM-1 (Cuff et al., 1999; Dejardin et al., 2002; Yoshida et al., 2002; Amé-Thomas et al., 2007; Vandenhoff et al., 2009a), as well as the homoeostatic chemokines CCL19, CCL21, and CXCL13 (Ansel et al., 2000; Luther et al., 2003). In addition, IL-7 and TRANCE induce the expression of LTαβ2 and generate a positive feed-back loop that sustains a continuous supply of signals between stroma and LTI cells granting maturation of the former (Ansel et al., 2000; Honda et al., 2001; Luther et al., 2003; Mebius, 2003).

MATURATION OF MESENCHYMAL CELLS: THE STROMACENTRIC VIEW

The general mechanism of SLO development, whereby LTI cells colonize lymphoid organ primordia, is similar among PP and LN anlagen (Yoshida et al., 2002; Randall et al., 2008; van de Pavert and Mebius, 2010; Cupedo, 2011). However, despite the obvious parallels there are also remarkable differences between the morphogenesis of these organs. Examples of such differential processes are provided by IL7/IL7R and TRANCE/TRANCE-R signaling. Thus, while IL7R signal is critical to PP development, as revealed by Bdr−/− mice, brachial, axillary, and mesenteric LN develop normally in these animals (Adachi et al., 1998; Yoshida et al., 1999; Luther et al., 2003). Furthermore, while in TRANCE−/− and Traf6−/− mice LN development is severely compromised, PP form normally in these mice (Dougall et al., 1999; Naito et al., 1999). Finally, the tyrosine kinase receptor RET also plays a differential role in LN and PP genesis. This is revealed by the absence of PP in Ret null embryos, which have seemingly normal LN anlagen development (Vega-Fernandes et al., 2007).

Interestingly, mesenchymal organizer cells from LN and PP also exhibit distinctive genetic features (Yoshida et al., 2002; Cupedo et al., 2004; Okuda et al., 2007). This genetic heterogeneity suggests that LTI cells may also provide different cues to hematopoietic cells. Nevertheless, it remains unclear whether the acquisition of such divergent genetic profiles are cell autonomous or derived from paracrine cellular interaction with different hematopoietic cell subsets.

The distribution of mesenchymal cells within lymphoid organs differs between PP and LN. In the intestine, stromal cells are distributed throughout the gut tissue that becomes colonized by highly motile hematopoietic cells between day E12.5 and E15.5. At this stage rare VCAM-1+ cells are detected in the gut wall (Adachi et al., 1997). However, by E16.5, VCAM-1+/-ICAM-1+/- clusters of stroma cells are clearly visible forming PP primordia (Adachi et al., 1997; Yoshida et al., 1999; Hashi et al., 2001; Vega-Fernandes et al., 2007). Conversely, LN invariably develop within lymph sacs, where ICAM-1+/-VCAM-1+ mesenchymal stromal cells initially surround endothelial cells and by E16.5 start to invade the endothelium to form a proper compartment of the anlagen (Okuda et al., 2007). Surprisingly, although lymphatic endothelial cells are essential to the correct formation of LN and lymphatic vasculature, they are dispensable for the initial aggregation of LTI and LTIb cells (Cupedo et al., 2004; Vandenhoff et al., 2008b; Benezech et al., 2010).

Interestingly, mounting evidence indicates that LTI cells are very heterogeneous. In PP genesis, VCAM-1+/-ICAM-1+/- organizer cells express ITgR, CCL19, and CXCL13 (Adachi et al., 1997; Yoshida et al., 1999; Hashi et al., 2001; Honda et al., 2001; Vega-Fernandes et al., 2007), and further analysis revealed that this cell population comprises VCAM-1+/-ICAM-1+/- and VCAM-1+/-ICAM-1+/- subpopulations (Okuda et al., 2007). Similarly, these populations were also identified in LN (Cupedo et al., 2004; Okuda et al., 2007; Benezech et al., 2010). The comparison of genetic expression between PP and LN VCAM-1+/-ICAM-1+/- cells shows that mesenteric LN LTIb cells have surface expression of TRANCE, whereas their PP counterparts lack the expression of this ligand (Cupedo et al., 2004; Okuda et al., 2007). Furthermore, microarray analysis revealed that their genetic signatures are distinct. Mesenteric LN stroma cells express significantly higher levels of cytokines and chemokines such as IL6, IL7, CCL11, CXCL1, and CCL11 (Okuda et al., 2007). Conversely, the homoeostatic chemokines CCL21, CCL19, and CXCL13 are more abundant in enteric stroma cells. Interestingly, genes implicated in morphogenesis, such as Meox2, Lhx8, and Prrx1, were significantly higher in mesenteric LN when compared to PP counterparts, yet their functional relevance in lymphoid organogenesis is unclear (Okuda et al., 2007).

In addition to previously described VCAM-1+/-ICAM-1+/- and VCAM-1+/-ICAM-1+/- stroma cells, another population of VCAM-1+/-ICAM-1+/-, expressing FGFRs but gp38/podoplanin and VEGFR3 negative was identified in LN (Benezech et al., 2010). Although, VCAM-1+/-ICAM-1+/- and VCAM-1+/-ICAM-1+/- cells have been described in PP, the existence of a VCAM-1+/-ICAM-1+/- counterpart remains to be investigated (Cupedo et al., 2004; Okuda et al., 2007). VCAM-1+/-ICAM-1+/- stroma cells express CCL21 and Tgfβ1 while VCAM-1+/-ICAM-1+/- express the highest levels of CCL2, CCL19, CCL21, Trace, and Il7, as compared with VCAM-1+/-ICAM-1+/-, confirming their greater potential to attract
The early priming events of enteric SLO: The LTI cell reign

In the intestine CD3+ CD4+ IL-7Rα+ LTI cells and VCAM-1+ ICAM-1+ stromal organizer cells cluster together with CD3+ CD4+ IL-7Rα+ cKit+ CD11c+ cells (Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007). Mice lacking CD3+ CD4+ IL-7Rα+ cKit+ CD11c+ cells failed to develop the receptor tyrosine kinase RET (Ret/−/−), expressed by this population do not develop PP (Veiga-Fernandes et al., 2007). Thus, CD3+ CD4+ IL-7Rα+ cKit+ CD11c+ cells are required in early phases of enteric lymphoid tissue formation and were named LTI cells (Fukuyama and Kiyono, 2007; Veiga-Fernandes et al., 2007). Supporting this concept, the RET ligand ARTN induces the formation of ectopic lymphoid structures, and LTI cells are the first hematopoietic cellular entity to cluster together with VCAM-1 expressing stroma cells (Veiga-Fernandes et al., 2007; Patel et al., 2012). Although, LTI cells are scarcely detected at very early phases of enteric organ formation, an extensive accumulation of LTI cells occurs subsequently to LTI cell aggregation (Patel et al., 2012). Interestingly, LTI cells respond unconventionally in trans to all RET ligands, reducing their motility upon contact with mesenchymal cells, in an adhesion-dependent manner (Patel et al., 2012). Furthermore, while Ccl19, Ccl21, and Cxcl13 chemokine expression is not required in this early triggering phase, VCAM-1 blockage results in a profound reduction of cell clustering efficiency, indicating that subsequent up-regulation of VCAM-1 in stroma organizer cells is essential to recruit and retain the first LTI cells (Patel et al., 2012). Thus, in opposition to the LTI action mechanism, where chemokines and LTI/LTI cell interactions are key (Hashi et al., 2001; Finnke et al., 2002; Luther et al., 2003; Ohl et al., 2003), LTI cells act at very early phases—determining early maturation of enteric mesenchymal cells in a RET-dependent, chemokine-independent manner (Patel et al., 2012). Strikingly, in agreement with previous reports in the LN, the initial induction of VCAM-1 expression in enteric stroma cells might not rely on the engagement of LTIβR, since RET ligand stimulation does not up-regulate LTI on LTI cells and blockage of LTIβR signaling does not impair VCAM-1 induction on stromal cells (Patel et al., 2012). Thus, we would like to propose that PP development is a multi-step, multi-cellular process relying on an initial RET-dependent and adhesion-dependent interaction between LTI and mesenchymal cells, which result in stroma cell priming, ultimately leading to efficient LTI cell recruitment (Figure 1). Although, CD11c+ cells have been detected in anlagen LN, Ret/−/− mice develop peripheral LN (Veiga-Fernandes et al., 2007). Thus it remains unknown whether LTI cells are also implicated in early stroma cell priming of LN. LTI cells have been phenotypically characterized. These cells present some features of dendritic cells, expressing CD11c, CD11b, and MHC class II, but lack DEC205 and express NK1.1 and Gr-1 (Veiga-Fernandes et al., 2007). Thus, it would be very interesting to understand the precursor-product relationship between LTI cells and other cell lineages. Finally, it would be exceedingly exciting to determine whether LTI and RET responses may also initiate enteric cryptopatches or lymphoid tissue induced in chronic inflammation.

CONCLUDING REMARKS

Over the last two decades, remarkable findings have consolidated our knowledge on lymphoid organogenesis. Despite differences between diverse lymphoid organs, we can now appreciate that recruitment of fully functional LTI cells is central in LN and PP organogenesis and that, upon their arrival, an intimate and productive cross-talk is established with...
FIGURE 1 | Model of Peyer’s patch development. Peyer’s patch development relies on a multi-step, multicellular process. Step 1: Ret-dependent, adhesion-dependent interaction between LTin and mesenchymal cells, results in stroma cell priming and VCAM1 induction (immature LTi cell). Step 2: retention of resident LTi cells through a VCAM1 mediated process; LTi/LTβ interaction (mature LTi cell). Step 3: Positive feedback loop generating fully mature LTi cell and additional LTi cell recruitment and retention into the primordium.

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Complexes of cytokine and TNFα are required for lymphoid system development, as well as for the initiation and maintenance of lymph node formation. The enteric stroma, which is composed of mesenchymal cells and stromal fibroblasts, plays a crucial role in the development and maintenance of lymphoid organs. Indeed, the enteric stroma is essential for the proper development of lymphoid organs, including lymph nodes and Peyer’s patches.

The enteric stroma is composed of a heterogeneous population of cells, including fibroblasts, immune cells, and non-immune cells. The stromal fibroblasts are the main cell type responsible for the production of extracellular matrix (ECM) components, which provide a physical scaffold for the development of lymphoid organs. The ECM components, such as collagen and laminin, are essential for the proper organization of lymphoid organs.

The enteric stroma also plays a role in the regulation of lymphocyte traffic and homing. Lymphocytes, which are attracted to the stromal fibroblasts by chemokines, are guided to their specific lymphoid organs by the ECM components. The ECM components also provide a physical barrier to prevent the escape of lymphocytes from the lymphoid organs.

Moreover, the enteric stroma is involved in the regulation of lymphocyte differentiation, proliferation, and survival. The stromal fibroblasts produce cytokines, such as transforming growth factor-β (TGF-β) and interleukin-6 (IL-6), which are essential for the differentiation and survival of lymphocytes.

In conclusion, the enteric stroma is a critical component of the lymphoid system, playing a vital role in the development and maintenance of lymphoid organs. The enteric stroma, through its production of ECM components and cytokines, provides a physical and functional scaffold for the development and maintenance of lymphoid organs.