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Organogenesis of peripheral lymphoid organs

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Summary: The discovery that lymphotoxin α (LTα) knockout mice lack peripheral lymphoid tissues reformed the study of organogenesis of peripheral lymphoid tissues from a research field that was solely descriptive and dependent on histological methods to one requiring all modern technologies. The concepts of inducer cells for organogenesis of peripheral lymphoid tissues as a separate hematopoietic lineage and of mesenchymal organizer cells have been established through this progress. These discoveries led to the comprehension of the basic framework of the events during organogenesis of peripheral lymphoid tissues. However, many important questions remain unanswered. This review discusses those questions which have arisen from our studies on the organogenesis of Peyer's patches.

Introduction

Over the last 10 years, since the discovery that the lymphotoxin α-deficient (LT$\alpha^{-/-}$) mouse lacks peripheral lymphoid tissues, our understanding of the organogenesis of lymph nodes (LNs), Peyer's patches (PPs), and nasal tract-associated lymphoid tissues (NALT) has made remarkable progress. As a consequence, a number of plausible scenarios that can explain most of the observations concerning these processes have been published (1–7). It should be noted, however, that inconsistencies are still present among the scenarios, and many questions remain unanswered. The major aim of this review is to elucidate the important questions for future investigation.

A scenario for the initial step of organogenesis of peripheral lymphoid organs

Our scenario for organogenesis of PPs is presented in Fig. 1. While some modifications have been made from the initial model (7, 8), the basic framework of the latest version is almost unchanged from the initial one. In the current model, the earliest step of PP organogenesis is viewed as a process to establish two mutually interacting cellular components in the
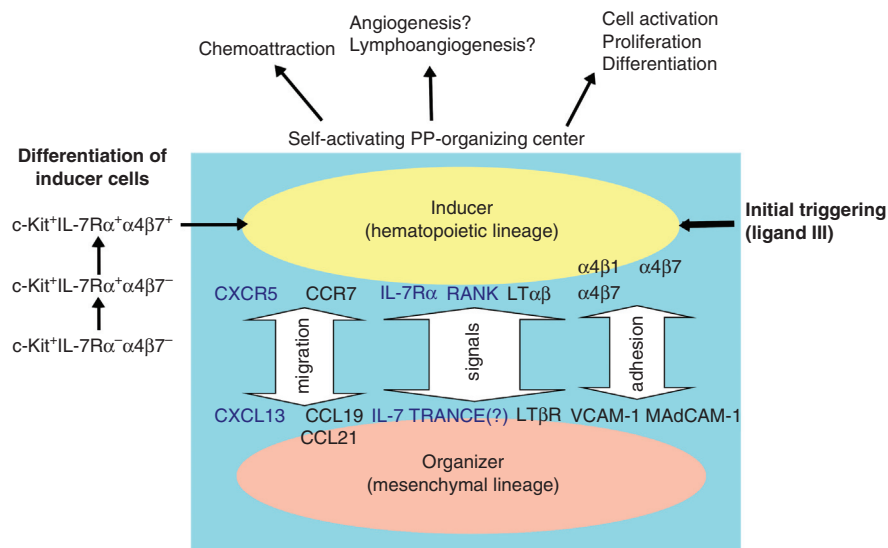
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Fig. 1. Our scenario for the initial step for Peyer's patch (PP) anlagen formation. PP anlagen are formed where the inducer cells and organizer cells are assembled. The inducer cells are differentiated from IL-7Rα⁺c-Kit⁺ progenitors in fetal liver through the process described in the left side of this figure. Differentiated cells migrate to the PP anlagen. The inducer and organizer cells are complementary in many aspects, expressing signal and adhesion molecules as listed in this figure, which support mutual interaction. Namely, LTαβ expression in the inducer cells is induced either by the ligand for IL-7Rα or receptor activator of nuclear factor κB (RANK), and the expression of IL-7 and CXCL13 molecules on the organizer cells is activated by LTβR signal. In addition to those molecules, expression of vascular cellular adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the organizer cells is induced by LTβR signal. Although the organizer cells express IL-7, IL-7 is not an absolute requirement for triggering the process of PP organogenesis, as PPs develop in IL-7$^{-/-}$ mouse. We speculate the presence of the third ligand for IL-7Rα, which remains to be characterized. Thus assembled, the two mutually stimulating cellular components form an autonomously activating region that may serve as the organizing center for subsequent processes of PP organogenesis. In addition to sustained production of chemokines and cytokines that are involved in chemoattraction and activation of various cell lineages in or in the vicinity of PP anlagen, we speculate that the molecules involved in angiogenesis and lymphoangiogenesis may also be produced in this organizing center.

PP anlagen. One component is the CD45⁺ cells that we termed PP inducers (9), and the other is mesenchymal cells that we termed PP organizers (10). Previous studies indicated that, like other hematopoietic cell lineages, the progenitors of the inducer cells are generated in the fetal liver and migrate to the regions where PPs and LNs develop (11, 12). As the organizer cells belong to the mesenchymal cells, it is unlikely that they are recruited by migration. Hence, we speculate that the organizer cells are induced by various stimuli either from the inducer cells or other cellular components in the anlagen. The PP inducer is characterized by its expression of molecules such as interleukin-7Rα (IL-7Rα) LTαβ, and CXCR5, whose involvement in PP organogenesis has been established by gene knockout (KO) studies (13–19). While their role in this process is not clear, CD4 and α4β7 integrin are surface markers that also have been used to characterize this population (12, 20). Likewise, PP organizers express such molecules as IL-7, LTβR, and CXCL13 (BLC) that are complementary to the molecules on the PP inducers (Fig. 2, 10). Moreover, vascular cellular adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MadCAM-1) that interact with α4β7 integrin also are expressed in PP organizers (10).

Concerning the relationship among the signals expressed in the two compartments, it has been shown that IL-7Rα stimulation induces the expression of LTαβ of inducer cells (10). LTβR stimulation in turn induces CXCL13 (10) and IL-7 (Fig. 2) production of organizer cells (10), which can stimulate the inducer cells. Once these two components are assembled in the same place, they mutually stimulate each other, thereby forming a unit that can maintain the active state autonomously. This active region expresses molecules that are involved in further progression of PP organogenesis. Because of the mutually stimulating ability of the two components, the region in which they are assembled expands autonomously. For instance, once the PP organizer cells are activated by LTαβ on the PP inducer, it emanates CXCL13 that in turn attracts PP inducer cells to the activated region. As a consequence, the LTαβ concentration in this activated region increases by accumulation of inducer cells, as the activated PP organizer cells emanate a number of molecules that can act on the PP inducer cells to express LTαβ on their surface. Hence, even starting from a small number of inducer and organizer cells, the active region can expand autonomously by recruiting more and more inducer and organizer cells, until those cells become

A

B
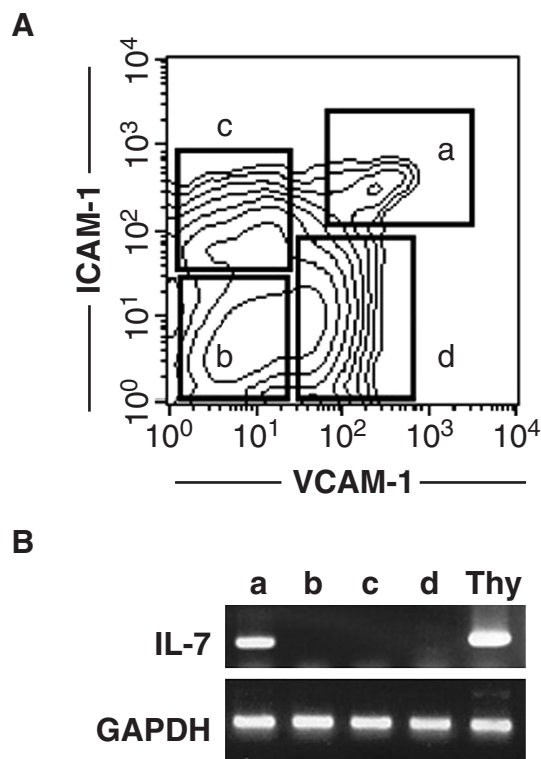
Fig. 2. **Expression of interleukin-7 (IL-7) mRNA in VCAM-1$^+$ICAM-1$^+$ cells.** (A) The cells dissociated from E17.5 embryos were double stained by antibodies to vascular cellular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and the stained cells were fractionated into VCAM-1/ICAM-1-double positive (A), -double negative (B), ICAM-1-single positive, (C) and VCAM-1-single positive (d) fractions. (B) Reverse transcriptase-polymerase chain reaction analysis of transcripts encoding IL-7 in each fraction was performed and presented together with that of thymus cells (Thy). IL-7 is expressed only in the fraction that corresponds to the organizer cells.

unavailable. Accumulating evidence strongly indicates that similar components are also present in the LN anlagen, though the molecules involved are different in LNs and PPs.

Accepting that the establishment of this mutually interacting unit is the goal for the initial step during PP organogenesis and probably also for LN organogenesis, the remaining questions are how it is initiated and how it gains the higher order architecture found in mature lymphoid tissues.

The first step triggering Peyer's patch organogenesis

Two important questions remain unanswered concerning the initial step that triggers PP organogenesis. One question concerns the first signal that triggers PP organogenesis, and the other concerns the mechanisms underlying how the positioning of PP is determined.

The ligand for interleukin-7Rα involved in Peyer's patch organogenesis

As IL-7Rα signaling induces LTαβ expression of PP inducers and LTβR signaling induces IL-7 expression in PP organizers (Fig.2), either IL-7Rα or LTβR stimulation can be the first event for triggering PP organogenesis. As clearly indicated by previous studies, LTαβ expression of PP inducers can also be induced by receptor activator of nuclear factor κB (RANK) (20, 21); there is a possibility that other molecules play a role in triggering the initial event for PP organogenesis. However, PP anlagen are formed normally in RANK$^{-/-}$ mice (22–24). Hence, RANK and CXCR5 may not be absolute requirements for triggering the PP organogenesis. While it is difficult to rule out the presence of yet unknown signals, we postulate that LTβR signaling or IL-7Rα signaling is the event initiating PP organogenesis.

One unresolved question concerning this process is the nature of ligand that stimulates IL-7Rα during PP organogenesis. In B- and T-cell genesis, IL-7 is the major ligand for IL-7Rα, as IL-7$^{-/-}$ and IL-7Rα$^{-/-}$ mice have a largely similar phenotype. In contrast, PP anlagen formation is defective in IL-7Rα$^{-/-}$, but not IL-7$^{-/-}$, mice. As shown in *Fig.* 3, VCAM-1$^+$ PP anlagen were detected in the guts of E18 IL-7$^{-/-}$ embryos as well as normal embryos, whereas none were detected in IL-7Rα$^{-/-}$ mice. Hence, with regard to PP anlagen formation, IL-7 cannot be the sole ligand for IL-7Rα. In addition to the formation of PP anlagen, further progression of PP organogenesis occurs in the absence of IL-7. *Fig.* 4 presents PP of normal and IL-7$^{-/-}$ mice at 14 days after birth. While the number of mature lymphocytes is reduced in IL-7$^{-/-}$ mice, both B and T cells are present, and the segregation of T and B cells as well as the formation of peanut agglutinin-positive (PNA$^+$) follicles was found in IL-7$^{-/-}$ mice (*Fig.* 4). These results imply involvement of other ligands for IL-7Rα for PP organogenesis. While thymic stromal lymphopoietin (TSLP) is another known ligand that binds to IL-7Rα (25), recent studies suggested strongly that its involvement in PP organogenesis is unlikely (26, 27). We have demonstrated that formation of PP anlagen is defective in common (c) γ$^{-/-}$ and Janus kinase 3-deficient (JAK3$^{-/-}$) mice as well as IL-7Rα$^{-/-}$ mice (13). This finding indicates that the IL-7Rα signal for PP organogenesis is transmitted through cγ/JAK3 pathway. In contrast, the signal transduction pathway of TSLP was recently characterized in detail and demonstrated to be independent of cγ and of JAK3 (27, 28) but required IL-7Rα and a newly identified TSLP receptor (26, 27). These observations indicate the existence of the third ligand (ligand III) for IL-7Rα for PP organogenesis, which is different from IL-7 and TSLP. Hence, the
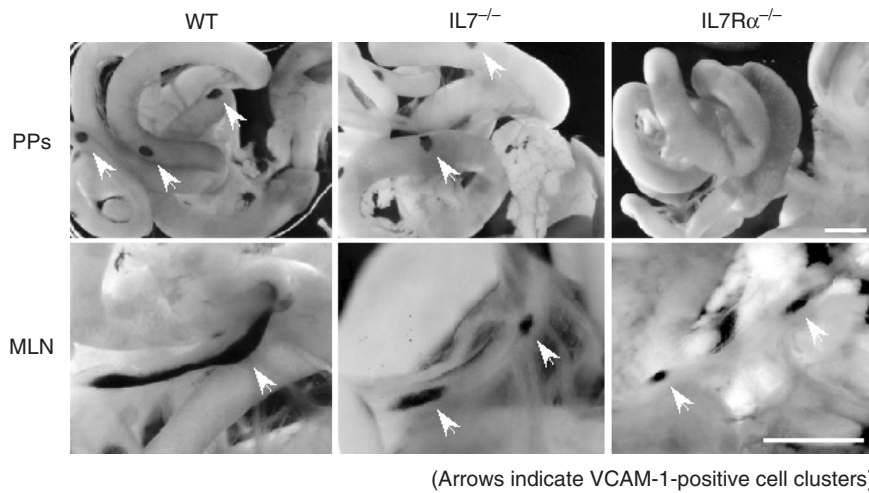
Fig. 3. **Formation of VCAM-1$^+$ Peyer's patch (PP) anlagen in IL-7$^{-/-}$ mice.** Vascular cellular adhesion molecule-1 (VCAM-1) expression is detected by the whole-mount immunostaining of intestines and mesenteric regions of E18 normal, IL-7R$\alpha^{-/-}$, and IL-7$^{-/-}$ embryos. VCAM-1 expression was detected in the mesenteric region of all strains of mice. This indicates that the formation of LN anlagen occurs normally in the absence of IL-7 or IL-7Rα. VCAM-1$^+$ PP anlagen are not formed in IL-7$\alpha^{-/-}$ embryos, whereas they develop normally in IL-7$^{-/-}$ mice.

(Arrows indicate VCAM-1-positive cell clusters)

identification of ligand III is essential for the analysis of the initial step of PP organogenesis.

Positioning of Peyer's patch anlagen

Variable numbers of PPs, ranging from 5 to 12, are found in the intestine of adult mice. This variation suggests that the site where a PP is formed may not be directed by a strict developmental program. As discussed in the preceding section, either LT$\alpha\beta$ or ligand III can be the initiator of the process. However, it is more likely that ligand III is the initial signal, as LT$\alpha\beta$ cannot constitute a regional signal due to the diffuse distribution of IL-7Rα^+ cells that are the sole source of LT$\alpha\beta$ in the developing intestine (12). Moreover, it was recently reported
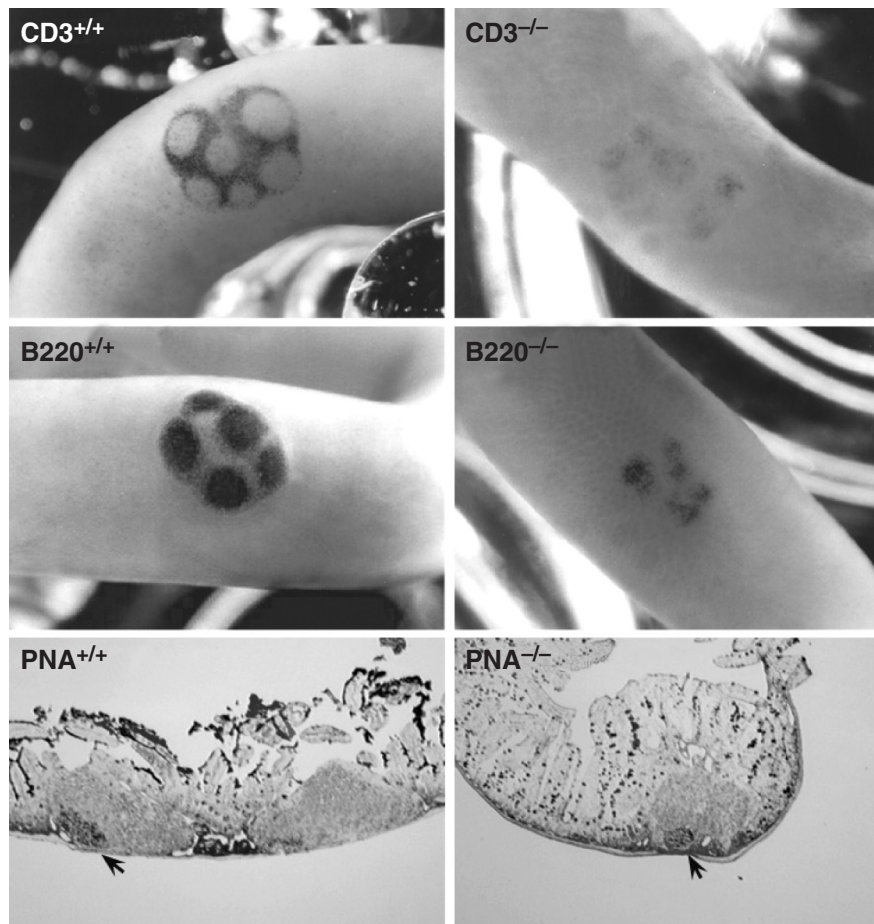
Fig. 4. **Peyer's patch (PP) formation in the absence IL-7.** PP formation is inhibited completely in IL-7R$\alpha^{-/-}$ mice (13). In contrast, PP anlagen are formed normally in IL-7$^{-/-}$ embryos (*Fig.* 2). Moreover, anlagen in IL-7$^{-/-}$ mice develop to the mature type PP with segregation of B and T cells as well as PNA$^+$ germinal centers (right panels). Compared to the PP of the control mouse (left panels), the numbers of B and T lymphocytes detectable in PP are severely reduced.

that CD3$^-$CD4$^+$ cells injected systemically to CXCR5$^{-/-}$ neonates could generate PPs *de novo* (3). As it is unlikely that LTαβ on the systemically injected inducer cells can form regional signals, this result also argues for the possibility that the positioning of a PP is determined by the regional expression of ligand III.

One could speculate two possibilities for the mechanism regulating the regional expression of ligand III. The first possibility is that the program regulating organogenesis of the gastrointestinal tract directs the expression of ligand III. The regional specificity of the gastrointestinal tract, from the esophagus to the rectum, is determined by the differential expression of HOX genes (29). Moreover, it has been suggested that the positioning of the associated organs, such as liver and pancreas, is determined by the same mechanisms. Thus, it is pertinent to think that the same program directs the positioning of PPs. One difficulty is, however, explaining the large individual variation in the number of PPs. Such a variation is not the case for other gut-associated organs.

The other possible mechanism is that the positioning of PPs is determined reactively as a result of a self-organizing chemical system in the gut, which spontaneously generates spatial patterns in the concentration of some of the molecular components regulating ligand III. If one supposes two diffusible molecules, A and B, that interact with each other as illustrated in *Fig.* 5, a stable repetitive wave is generated for the concentration of the molecules (30, 31). Indeed, this rule has been implicated in the process of establishing repetitive patterns, such as stripes on fish (32). The first PP anlage appears at the border of duodenum and ileum, and thereafter new anlagen are generated successively one by one towards lower intestine at a relatively regular interval, although the final

number of PPs is variable. This generative and repetitive pattern found in the PP positioning leads us to speculate that the location of PPs may be determined by the reaction-diffusion system.

Timing of PP anlage formation

A striking observation in the study of PP and LN organogenesis is that a single injection of antagonistic reagents against IL-7Rα or LTαβ at a specific time point can permanently block the organogenesis of PPs and LNs, respectively (9, 33). As this method is applicable for any strain of mice including transgenic and gene KO mice, it has become a standard method to manipulate PP and LN organogenesis. Indeed, recent studies employed this method to compare the immune response in the presence and absence of LNs or PPs (34, 35).

In PP organogenesis, we have demonstrated that the irreversible block of PP organogenesis by anti-IL-7Rα monoclonal antibody (mAb) occurs only during a narrow time window, from 12 to 14 days of gestation (E12–E14) (9). Injection of anti-IL-7Rα mAb at a later stage had no suppressive effect on PP organogenesis. For example, PPs on the upper part of gut remained unaffected by injection at E15, and all PPs developed normally with the injection later than E16. This result indicates that for PP genesis, IL-7Rα signaling is essential for triggering the process, but it continues normally without IL-7Rα signaling after the assembly of inducer and organizer cells in PP anlagen.

Likewise, it was demonstrated that a single injection of blockers of LTβR signal irreversibly inhibited the formation of LNs, but the location of LNs affected by the treatment varied according to the timing of injection (36). This result is consistent with the notion that triggering of LN genesis also occurs during a narrow time window.

The process of PP genesis is inhibitable by the blockers of LTβR signaling, even after the blocker of IL-7Rα has no effect (36, 37). This finding suggests either the involvement of multiple signals in the induction of LTαβ of inducer cells or involvement of additional cells that express LTαβ. We prefer the former possibility, as it was shown that multiple signals, including tumor necrosis factor-related activation-induced cytokine [TRANCE, also known as RANK ligand (RANKL)], tumor necrosis factor-α (TNF-α), or even thrombopoietin, can induce LTαβ expression of the inducer cells (21). Thus, after the IL-7Rα-dependent assembly of inducer and organizer cells has occurred, a more complex molecular network is established for maintaining the LTαβ expression, thereby stabilizing the inducer activity that is a requirement for the
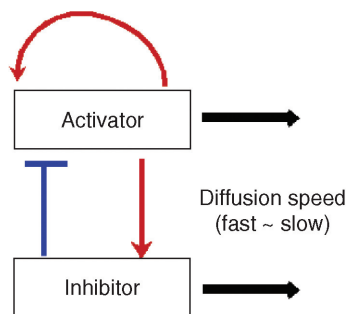
Fig. 5. The conditions required for the reaction-diffusion system. When two diffusible molecules, where one is an activator of itself as well as the other and the second is an inhibitor, react to each other in a restricted field, various periodic waves in the concentration of these molecules are spontaneously formed. For details, see the review by Kondo (31).

aforementioned process. Which molecules are available from the organizer remains to be elucidated.

It was recently shown that the injection of CD3$^-$CD4$^+$ inducer cells from fetal livers of normal embryos into the CXCR5$^{-/-}$ neonates effectively restored the PP genesis in this mouse strain (3). As this restoration was blocked by anti-IL-7Rα mAb, this result demonstrated for the first time that the regional expression of the ligand for IL-7Rα, most likely ligand III, is maintained at least up to the neonatal stage and stimulates inducer cells to form PP anlagen *de novo*. It is interesting that the number of PPs that were induced by the neonatal injection was three times higher than that of normal mice. This increase in the PP number could be due to the fact that intestines of CXCR5$^{-/-}$ mice, which were used for the host, are hyperactive in terms of the activity of inducer stimulation. Indeed, the author presented some evidence for the presence of such a hyperactive state in the intestine of CXCR5$^{-/-}$ mice, although the underlying mechanisms are unknown. Five-fold more inducer cells are detected in the intestine of CXCR5$^{-/-}$ mice, and the numbers of VCAM-1$^+$ cells that may represent activated mesenchymal cells were higher in the CXCR5$^{-/-}$ mice (3).

An alternative explanation for the increased number of PP is that the number of the waves of the ligand III concentration generated by the reaction-diffusion mechanisms (described in the preceding section) increases along with gut development (*Fig.* 5). In some cases, extension of a reaction field leads to increase of a repetitive unit, as most clearly documented in the striped pattern of a fish species (32). Hence, this hypothesis for PP organogenesis is consistent with the reaction-diffusion system. As the number of PPs in the gut is determined before E15 in normal mice, the sustained increase of PP number in the CXCR5$^{-/-}$ mice, which lack PPs, suggests that formation of actual PP anlagen interrupts this reaction wave, thereby inhibiting further increase in the number of PPs. If this is the case, it may be further distinguished when inducer cells are injected earlier during embryogenesis. If the formation of the functional PP anlagen can inhibit the further increase of the regions capable of stimulating the inducers, the PP number may be normal with earlier injection of the inducer cells into CXCR5$^{-/-}$ embryos. In any case, final resolution of all these questions requires the identification of ligand III.

Role of LTαβ

In parallel with studies utilizing antagonistic reagents, agonistic reagents have also been used for restoring the lymphoid tissues of mice that lack these tissues (21, 36). Rennert *et al.*

(36) injected an agonistic mAb against LTβR to pregnant LTα$^{-/-}$ mice and succeeded in restoring LN genesis. Of particular interest is that this experiment demonstrated the timing when each set of LN genesis is triggered, as the antibody injection at different time points was effective in inducing growth of different sets of LNs. For instance, sacral and some mesenteric LNs require the mAb injection earlier than E13, while axillary LNs developed with the injection by E17. This result is consistent with the results using antagonistic reagents and also with scenario in *Fig.* 1, in which LTαβ signal plays a central role in the activation of organizer cells by inducer cells. However, a recent study by Kim *et al.* (23) suggests more complex molecular requirements for the inducer cells. In this study, they attempted to restore LN genesis of TRANCE (RANKL)$^{-/-}$ mice. While the rescue was attained by forced expression of the TRANCE transgene on the surface of T cells, injection of the agonistic mAb to LTβR that can rescue the LN genesis of LTα$^{-/-}$ mice could not do so for TRANCE$^{-/-}$ mouse. This result suggests that TRANCE induces multiple events, of which only some can be compensated for by the stimulation of LTβR. In this study, they measured the number of the CD3$^-$CD4$^+$ cells that were collected from the mesenteric region of TRANCE$^{-/-}$ or LT$^{-/-}$ mice, where LNs are present in other mouse strains, and found that these cells were reduced more in TRANCE$^{-/-}$ mice than LTα$^{-/-}$ mice. This finding was interpreted to show that TRANCE plays a role in the recruitment of inducer cells into LN anlagen, and the investigators proposed that the primary reason for the failure of LN organogenesis in the TRANCE$^{-/-}$ mice is due to insufficient accumulation of inducer cells in the LN anlagen. It is not clear, however, whether or not the number of CD3$^-$CD4$^+$ cells at the day of birth can reflect their proliferation at earlier stages when LN organogenesis is triggered. Moreover, whether or not CD4, which is used for the marker for the inducer cells, is stably expressed in the defective microenvironment is yet to be determined. It is interesting that forced expression of TRANCE on the T cells could correct the defect of TRANCE$^{-/-}$ mouse. For T cells to be involved in the recruitment of the inducer cells, the inducer cells should encounter the transgene-bearing T cells before their migration to LN anlagen. Considering the fact that only a small number of T cells are present in E13 embryos when LN organogenesis is triggered, where inducer cells can meet the TRANCE$^+$ T cells and whether TRANCE$^+$ T cells act directly on the inducer cells are the questions to be addressed in future studies. An alternative and simpler possibility is that the transgene is expressed at low level, though Kim *et al.* (23) failed to detect the transgene-expression in the CD3$^-$CD4$^+$ cells of neonatal mice.

Based upon results using the TNF receptor-associated factor-6 deficient (TRAF6$^{-/-}$) mice instead of TRANCE$^{-/-}$ mice, we proposed a simpler hypothesis (21). As both IL-7 and TRANCE can equally induce expression of LTαβ on the surface of the inducer cells, we proposed that the role of TRANCE in LN genesis is to induce LTαβ expression on the inducer cells rather than in inducing their migration to LN anlagen. In other words, we hypothesized that the role of RANK in LN genesis is equivalent to that of IL-7Rα in PP genesis. Consistent with this notion, we showed that injection of IL-7 into TRAF6$^{-/-}$ embryos could restore LN genesis. This result indicates that the IL-7Rα signal is virtually equivalent to the RANK signal in the activation of inducer cells in the LN anlagen. This result further suggests that a sufficient number of inducer cells should have already accumulated to the LN anlagen at the time of IL-7 injection in the absence of TRAF6. Our study showed that IL-7Rα$^+$ cells were clustered in the LN anlagen in TRAF6$^{-/-}$ mice. If all signal transduction pathways downstream of RANK pass through TRAF6, our result is clearly inconsistent with the hypothesis of Kim *et al.* (23) in that RANK signaling is essential for migration of the inducer cells. Hence, it would be important to compare the presence of IL-7Rα$^+$ cells in the LN anlagen between TRANCE$^{-/-}$ and TRAF6$^{-/-}$ mice. It is also important to examine whether or not soluble LTβR agonist can restore the LN genesis of TRAF6$^{-/-}$ mice, as it failed to do so in TRANCE$^{-/-}$ mice. If there are other signals involved in the activation of organizer cells by inducer cells in addition to LTαβ signal, we need to construct a more complex scheme for LN organogenesis than that described in *Fig. 1*.

Localization of inducer cells

It is interesting to note that LN genesis but not PP genesis of LTα$^{-/-}$ mice, which lack LNs and PPs, can be restored by systemically delivered soluble agonist to LTβR (36). Likewise, LN genesis of TRAF6$^{-/-}$ mice, which lack LNs, can be restored by soluble IL-7 that can induce LTαβ expression of the TRAF6$^{-/-}$ inducer cells (21), but soluble TRANCE, which can induce LTαβ expression of the IL-7Rα$^{-/-}$ inducer cells, failed to restore PP genesis in these mice (our unpublished observations). This difference in the feasibility of restoring the organogenesis by systemically delivered soluble agonists may reflect the fundamental difference in the mode of anlage formation of the two organs, though both processes share many common mechanisms. We think that the major cause of this difference may relate to the distribution pattern of the inducer cells. We have compared the distribution of inducer cells in various strains of mice and found that they are clustered in the LN anlagen of TRAF6$^{-/-}$ and LTα$^{-/-}$ mice, whereas they are diffusely distributed and do not form clusters in the intestine of LTα$^{-/-}$ and IL-7Rα$^{-/-}$ mice. These distribution patterns suggest that the inducer and organizer cells should be assembled in a focal region in order to trigger the organogenesis by a systemically delivered soluble activator, whereas the localized expression of activators is a requisite for triggering the organogenesis when inducer cells are distributed diffusely.

Evidence suggested that the localization of LN anlagen is determined before the migration of inducer cells. Based upon histological observations, it was suggested that LN anlagen are formed in the region where a vein and lymph sac are in close vicinity (38, 39). Indeed, we also observed that inducer cells migrate to this region through the venous endothelial layer. While studies of lymphatic vessels have been hampered by the difficulty in distinguishing lymphatic vessels from blood vessels, recent studies identified two molecules, Flt4 and Prox1, which are involved in the development of lymphatic system (40, 41). It was demonstrated that lymphatic vessels are differentiated from venous endothelial cells through a process in which Prox1 is involved in the differentiation from venous endothelial cells (42), and Flt4 is involved in the proliferation of lymphatic endothelial cells (43–45). Taking this current view into consideration, it is likely that LN anlagen are formed when the venous vessels bud out to form lymphatic vessels. Given that this is the case, it is reasonable to think that many regions that are surrounded by venous and lymphatic vessels are generated during this stage, when lymphatic vessels bud from the vein. Since inducer cells migrate to thus formed future LN anlagen even in LTα$^{-/-}$ or TRAF6$^{-/-}$ embryos, these regions should be equipped with the activity to attract inducer cells. Immunohistological analyses demonstrated that CXCL13 is indeed expressed in this region (our unpublished observation). How the budding of lymphatic vessels leads to the formation of active regions that express CXCL13 and probably TRANCE is an important issue for future investigation.

That the injection of both soluble agonist of LTβR as well as soluble IL-7 could induce LN development implies that there is no preference which components, inducer or organizer cells, should be stimulated first. This finding is consistent with our model for PP organogenesis, in which the inducer and organizer cells stimulate each other to form a self-activating unit. In this situation, the assembly of two components in the anlagen is the primary requirement for the progression of the organogenesis.

In contrast to the LN anlagen, where accumulation of inducer cells occurs before activation of the inducer and organizer cells, PP organogenesis has to be initiated in the field where inducer cells are distributed diffusely. In such a situation, localized expression of the activator, whether stimulation is of inducer cells or organizer cells, is essential for forming active anlagen. As discussed in the preceding section, once such a region is formed, it expands by recruiting more inducer cells.

Reversibility of the process

The anlagen of PPs and LNs at the initial stage of their development are regions in which various cell lineages are accumulated selectively, thereby demarcating anlagen from surrounding regions. The highly complex architecture found in the mature LNs and PPs, where each cell lineage is segregated into distinct regions, is not yet attained in the anlagen. Previous studies demonstrated that this complexity is acquired through a series of processes that are induced upon entry of mature lymphocytes in both an antigen-dependent and -independent manner (1, 5). In addition, we have demonstrated that some extent of complexity is established in SCID/SCID or RAG2 KO mice, which lack mature lymphocytes (46). This result indicates that the segregated regions that attract distinct subsets of lymphocytes have been established before the entrance of lymphocytes. Immunohistological staining showed that homogeneously distributed IL-7Rα^+ inducer cells become segregated to form regions that most likely develop into the primary follicles, thereby separating the future B-cell region from interfollicular spaces where T cells migrate. Moreover, the MAdCAM-1$^+$ high endothelial venules that are characteristic of the lymphoid follicle were detected in SCID/SCID mice. While not much is known concerning the underlying mechanisms regulating this early process, it is known that it is entirely dependent upon LT$\alpha\beta$ signal, as the antagonistic reagents injected during this stage could completely reverse this process (33,37). Through this treatment, PPs are permanently lost from the intestine. As IL-7Rα^+ inducer cells are still the major population expressing LT$\alpha\beta$, their involvement in the segregation of future lymphoid follicles in PP anlagen is highly likely. How LT$\alpha\beta$ on inducer cells can induce segregation of inducer cells themselves is yet to be investigated.

Anti-LT$\alpha\beta$ treatment, which could block PP genesis by day 3 after birth, failed to do so when injected at day 4(37, our unpublished observation). We could not detect a significant difference in the cellular organization of PP between day 3 and day 4 after birth. Nonetheless, this result indicates that there should be a structural basis that distinguishes day 3 PPs from that of day 4. As hematopoietic cells are more or less migratory and may not be able to contribute to stable tissue architecture, we speculate that this irreversibility would be attributed to the generation of non-hematopoietic cell components in the PP anlagen. It could either be a specific vascular system, a lymphatic system or appearance of new non-migratory mesenchymal or dendritic cells in the anlagen, but further studies are required.

Conclusion

In the last decade, molecular genetic studies revealed that many molecules are involved in the organogenesis of peripheral lymphoid organs. Most of these molecules have been assigned to the framework of a scenario with two cellular components, inducer and organizer cells, as the central unit for the organogenesis. In this article, we focus on the early steps during induction of PP anlagen before mature lymphocytes and antigens become involved in the process of organogenesis. While the scenario presented in *Fig. 1* can account for many observations concerning the organogenesis of peripheral lymphoid tissues, many questions remain unanswered.

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