結果、得られた２重変異 IL-15 Tg/T 細胞レセプターα鎖欠損マウスは、T 細胞レセプターα鎖を単独で欠損したマウスに比べて、腸炎の発症が早期より観察され、さらに重症遷延化することが明らかになった。重症腸炎発症２重変異マウスの病変部大腸粘膜より分離したββT細胞は IL-4 を主体とした Th2 型のサイトカインを発現し、また Bcl-2 の発現亢進を介して、病変部大腸粘膜に長期間にわたって生存・維持されることが明らかになった。また重症化腸炎発症 T 細胞レセプターα鎖欠損マウスの病変部大腸ββT 細胞は高いレベルの活性化 NK レセプター、NKG2D を発現していることが明らかになった。（８）Th2 型サイトカイン産生性 $NKG2D^{+}$ββT 細胞を腸管局所発現 MICA Tg/RAG2 欠損マウスに養子移入し、移入後の腸炎の重症度を RAG2 遺伝子単独欠損マウスと比較検討した。その結果、MICA 遺伝子を有する２重変異マウスにおいて腸炎の程度が軽減することが明らかになった。

D.　考察

近年、微生物感染、発がん物質などのストレス侵襲をうけて恒常性を逸脱した粘膜上皮において組織適合抗原に近縁の MICA の発現が亢進すること、また特定の抗原受容体を発現したγδ T 細胞がその機能発現に MICA を要求することが示されたことから、MICA が粘膜におけるホメオスタシスの逸脱マーカーとして粘膜免疫の機能発現に寄与していることが予想されていた。そこで分担研究者は、粘膜上皮限局 MICA トランスジェニックマウス、つまりストレスをたえずうけた腸内環境を再現したモデルを作成し、その粘膜免疫の動態を解析した。その結果、MICA が粘膜内リンパ球の発達を正に統御すること、またこの MICA の統御をうけた粘膜内リンパ球が難治性粘膜疾患（ハプテン誘導性大腸炎モデル）の発症を制御しうることを見出した。以上の成果より、MICA は粘膜におけるホメオスタシス逸脱マーカーとして、単に粘膜内リンパ球の機能を制御するのみならず、粘膜内リンパ球の発達をも統御する分子として粘膜における免疫学的恒常性の維持に包括的に寄与していることが明らかになった。

またヒトの潰瘍性大腸炎に酷似した自然発症慢性腸炎モデル T 細胞レセプターα鎖欠損マウスを用いて、慢性腸炎の重症遷延化に粘膜上皮より産生される IL-15 が重要な役割を果たしていることが明らかになった。IL-15 は組織常在リンパ球における活性化 NK レセプターNKG2D の発現を正に統御することが報告されており、T 細胞レセプターα鎖欠損マウスで観察される慢性腸炎の後期病変の形成および重症遷延化には、大腸粘膜浸潤ββT 細胞における IL-15/NKG2D を介したシグナル伝達系が重要な役割を果たすことが窺えた。また MICA Tg/RAG2 欠損マウスを用いた T 細胞移入腸炎誘発モデルの解析結果より、ストレス誘導性恒常性逸脱化シグナル MICA は、粘膜炎症の制御に寄与することが明確に示された。

E.　評価

１）達成度について　当初の目標としていた粘膜恒常性逸脱化分子 MICA のトランスジェニックマウスの作成を終え、MICA の粘膜免疫システムの構築と作動メカニズムにおける多彩なはたらきを明らかにすることができたことに加えて、MICA を利用したあらたな粘膜炎症アレルギー疾患の治療戦略の萌芽を得ることができ、たいへん満足している。

２）研究成果の学術的・国際的・社会的意義について　MICA Tg マウスを用いて、MICA が単にストレス逸脱化シグナルとして粘膜ホメオスタシスの維持に寄与しているのみならず、粘膜免疫機構の発生・分化を正に統御しうることを世界に先駆けて示したこと、さらに MICA を利用し

た新規のアレルギー免疫疾患制御法を示し得たことは、極めて国際的に科学的社会的価値が高いといえる。

3） 今後の展望について　MICA 分子を介した粘膜制御の機構を分子のレベルでさらに詳細に解明する必要がある。また腸管以外の粘膜や皮膚に好発するアレルギー免疫疾患への応用の可能性を仔細に評価する必要がある。

4） 研究内容の効率性について　ほぼ順調に研究成果が得られており、問題ないと考えている。

F.　結論

ストレス誘導性恒常性逸脱化シグナル MICA は、IL-15/NKG2D シグナルを介した粘膜炎症アレルギー疾患の、治療と制御に有用なことが明らかになった。

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H. 知的所有権の出願・取得状況

１特許取得　なし

２実用新案登録　なし

３その他　なし

厚生労働省科学研究費補助金（免疫アレルギー疾患予防・治療研究事業）
（分担）研究報告書

上気道粘膜における好酸球浸潤と難治化に関する研究

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研究要旨：本研究では、好酸球浸潤の機序およびその修飾因子を明らかにすることを目的として、好酸球の接着因子であるVCAM-1および強力な血管透過性因子であるVEGFの上気道培養細胞からの産生、さらに各種炎症性サイトカインの上気道粘膜からの産生について検討した。上気道粘膜への好酸球浸潤には、アレルギーのみならず感染や慢性炎症、低酸素状態など様々な要因が関与し、高度の粘膜浮腫や腫脹そして難治化をもたらすと考えられる。

A. 研究目的

アレルギー性鼻炎を中心とする上気道アレルギー疾患においては、局所粘膜への好酸球浸潤が特徴的であり、その程度が病態の重症そして難治化に関与することが知られている。そこで、本研究では、好酸球浸潤の機序およびその修飾因子を明らかにすることを目的として、好酸球の接着因子であるVCAM-1および強力な血管透過性因子であるVEGFの上気道培養細胞からの産生、さらに各種炎症性サイトカインの上気道粘膜からの産生について検討した。

B. 研究方法

鼻粘膜そして鼻茸から血管内皮細胞および線維芽細胞を分離培養し、これを継代培養したものを実験に供した。これら培養細胞をTNFα、IL-4、LPSで一定時間刺激し、VCAM-1、Eotaxin 、VEGFの産生を観察した。さらに、アレルギー性鼻炎患者から、中耳貯留液、鼻咽腔液、鼻分泌液を採取し、IL-8、VEGF濃度を測定した。また、ゲルシフトアッセイにより、転写活性因子であるNF-κBの活性化を観察した。

（倫理面への配慮）

本研究の実施に際しては当施設の臨床倫理委員会に承認を得た。そして、研究対象者に本研究内容ならびに不利益や危険性の無いことを説明し文書にて同意を得た。

C. 研究結果

1）鼻培養細胞におけるVCAM-1、Eotaxinの発現：TNFα、IL-4刺激によって鼻粘膜血管内皮細胞および鼻線維芽細胞から可溶性VCAM-1そしてEotaxinが産生され、その濃度はTNFαとIL-4を同時に加えることによってさらに高値を示した。TNFα刺激によるVCAM-1の発現はNF-κBの活性化と相関したが、IL-4刺激ではNF-κBそしてAP-1の活性化は認められなかった。

2）鼻培養細胞におけるVEGFの発現：鼻線維芽細胞をエンドトキシンで刺激するとその培養上清中のIL-8、RANTESの産生が有意に上昇し、低酸素下の培養ではVEGF産生のみ有意に上昇した。一方エンドトキシンと低酸素の両者で刺激すると、VEGF産生においては相乗効果が認められた。さらにこのVEGF産生はステロイドそして抗

アレルギー薬によって有意に抑制された。

3）外分泌液中サイトカイン：鼻咽腔液中からはすべての症例でIL-8が検出され、その濃度を鼻咽腔からのインフルエンザ菌検出の有無で比較すると、インフルエンザ菌検出例は非検出例と比較して有意に高値であった。VEGFは鼻分泌液ならびに中耳貯留液からも検出され、鼻分泌液ではアレルギー性鼻炎、アレルギー性鼻炎を合併する副鼻腔炎、化膿性副鼻腔炎の順で高値を示した。中耳貯留液中のVEGFは粘液性貯留液のほうが漿液性貯留液よりも高値であり、乳突蜂巣の発育が不良なものでより高値を示した。

C. 考察

上気道感染症の重要な起炎菌のひとつであるインフルエンザ菌に含まれるエンドトキシンは鼻咽腔や鼻腔における好酸球走化因子であるEotaxinやRANTESの産生を促し、またTNF-α産生を誘導することによってVCAM-1の発現を亢進し、上気道粘膜のアレルギー性炎症や好酸球性炎症を修飾していることが示唆される。さらに閉鎖腔である中耳腔や副鼻腔においてはアレルギー性炎症によってもたらされる換気不全によってもVEGFが産生される。最近、好酸球がVEGFを産生し、その受容体も存在することが証明されており、上気道粘膜での好酸球浸潤の悪循環が示唆される。

D. 評価

1）達成度について

本研究の目的とする好酸球浸潤に関わる因子の役割をin vitroで実証することは概ね達成しえた。また、いずれも間接的な成績であり、好酸球浸潤への直接的な関与を証明するには至らなかった。

2）研究成果の学術的・国際的・社会的意義について

好酸球浸潤の機序を明らかにすることは、アレルギー性鼻炎のみならず、好酸球性中耳炎や好酸球性副鼻腔炎などにより重篤な難治性疾患の病態解明においても急務とされる。また、これらの好酸球増多性疾患は国際的にも問題視されており、本研究の学術的・国際的・社会的意義は大きい。

3）今後の展望について

今回の研究でその重要性が確認された種々の因子が実際の好酸球浸潤にどれくらいの比重をもって作用するのか、そしてin vivoにおいてこれらの因子がどのような関連性をもって好酸球浸潤を促進あるいは抑制するかを明らかにしたい。

4）研究内容の効率性について

鼻線維芽細胞の培養や上気道粘液の採取は極めて容易であり、研究は効率的に実施された。一方、血管内皮細胞の培養は難しく決して効率性は高くなかった。今後は培養株を用いての比較研究が必要と思われる。

E. 結論

上気道粘膜への好酸球浸潤には、アレルギーのみならず感染や慢性炎症、低酸素状態など様々な要因が関与し、高度の粘膜浮腫や腫脹そして難治化をもたらすと考えられる。

F. 健康危惧情報（総括研究報告書を参照）

G. 研究発表

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黒野祐一：シンポジウム「粘膜免疫機構を駆使したアレルギー予防・治療戦略へ向けての最近の展開－上気道粘膜免疫機構と治療戦略－」（第 54 回日本アレルギー学会）

Yuichi Kurono. Expression of VCAM-1 in Nasal Mucosa and the Role in Eosinophilic Accumulation. (10th International Rhinologic Society Congress)

H. 知的所有権の出願・取得状況

なし

研究成果の刊行に関する一覧

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研究成果の刊行物・別刷り

NALT- VERSUS PEYER'S-PATCH-MEDIATED MUCOSAL IMMUNITY

Hiroshi Kiyono and Satoshi Fukuyama

Abstract | Recent studies indicate that the mechanism of nasopharynx-associated lymphoid tissue (NALT) organogenesis is different from that of other lymphoid tissues. NALT has an important role in the induction of mucosal immune responses, including the generation of T helper 1 and T helper 2 cells, and IgA-committed B cells. Moreover, intranasal immunization can lead to the induction of antigen-specific protective immunity in both the mucosal and systemic immune compartments. Therefore, a greater understanding of the differences between NALT and other organized lymphoid tissues, such as Peyer's patches, should facilitate the development of nasal vaccines.

MICROFOLD (M) CELLS Specialized antigen-sampling cells that are located in the follicle-associated epithelium of the organized mucosa-associated lymphoid tissues. M cells deliver antigens by transepithelial vesicular transport from the aero-digestive lumen directly to the subepithelial lymphoid tissues of nasopharynx-associated lymphoid tissue and Peyer's patches.

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The mucosal immune system is responsible both for mediating the symbiotic relationship between the host and endogenous microorganisms (commensal bacteria), and for functioning as a first line of physical and immunological defence against invading pathogens[1]. Through innate and acquired immunity, the mucosal immune system maintains immunological homeostasis along the vast expanse of the epithelial surface area, ranging from the oral and nasal cavities to the respiratory, intestinal and genito-urinary tracts.

The initiation of antigen-specific immune responses occurs at special 'gateways', which comprise MICROFOLD (M) CELLS located in the epithelium overlying follicles of the mucosa-associated lymphoid tissues (MALT). These contain all of the immunocompetent cells that are required for the generation of an immune response (that is, T cells, B cells and antigen-presenting cells). Peyer's patches, in the gut, and nasopharynx-associated lymphoid tissue (NALT) — two of the main components of MALT — are important inductive tissues for the generation of mucosal immunity through the ingestion and inhalation of antigen in the intestinal and respiratory tracts respectively[1] (FIG. 1). The COMMON MUCOSAL IMMUNE SYSTEM (CMIS) connects these inductive sites (that is, the Peyer's patches and NALT) with effector sites (such as the lamina propria of the intestinal and respiratory tracts, and glandular tissues) for the generation of antigen-specific T helper 2 (T_H2)-cell-dependent IgA responses, and T_H1-cell- and cytotoxic T lymphocyte (CTL)-dependent immune responses, which function as the first line of defence at mucosal surfaces[1,2].

In this review, we discuss three issues concerning the biology of the NALT immune system: first, we focus on the unique characteristics of its tissue genesis compared with that of Peyer's patches; second, we examine the immunological function of NALT; and third, we discuss manipulation of the NALT immune system to develop mucosal vaccines.

Distinct features of NALT organogenesis

Despite the functional similarity of NALT and Peyer's patches in terms of their role as mucosal inductive sites, their programmes of lymphoid organogenesis are distinct. On the basis of recent studies, the unique characteristics of NALT development compared with those of Peyer's patches have become clear in terms of both kinetics and cytokine requirements.

Chronological development. In normal mice, NALT is a bell-shaped tissue that is characterized by an accumulation of lymphoid cells. In contrast to the HIGH ENDOTHELIAL VENULES (HEVs) of Peyer's patches, which express mucosal vascular addressin cell-adhesion molecule 1 (MADCAM1) (REF. 3), NALT-associated HEVs express peripheral-node addressin (PNAD). Vascular cell-adhesion molecule 1 (VCAM1) has been

shown to be associated with the tissue genesis of Peyer's patches, because a cluster of VCAM1$^+$ stromal cells occurs at the site of Peyer's-patch development on day 15.5 after coitus[4]. To determine when NALT develops, we used immunohistochemistry to analyse PNAD expression in wild-type mice of various ages. NALT formation was not observed during embryogenesis or in newborn mice[5] (FIG. 2), whereas Peyer's patches were already present in the embryo as dome-shaped lymphoid tissues[6]. Instead, PNAD$^+$ HEVs with associated lymphocytes were first detected bilaterally in nasal tissue at 1 week after birth, and the complete formation of bell-shaped NALT (including lymphoid cells) was not observed until 5–8 weeks after birth[5] (FIG. 2). In rats, the development of NALT is also observed postnatally as a small accumulation of lymphoid cells[7]. These findings indicate a prenatal initiation of lymphoid organogenesis for Peyer's patches and a postnatal initiation for NALT. An intriguing possibility is that the NALT-genesis programme is triggered after birth through stimulatory signals that are provided by environmental antigens and mitogens. This view is supported by the finding that nasal administration of cholera toxin, a well-known mucosal immunogen with adjuvant activity, resulted in the acceleration of NALT organogenesis and the development of the bell-shaped lymphoid tissue[5]. Therefore, environmental stimulation might be essential for NALT organogenesis, although

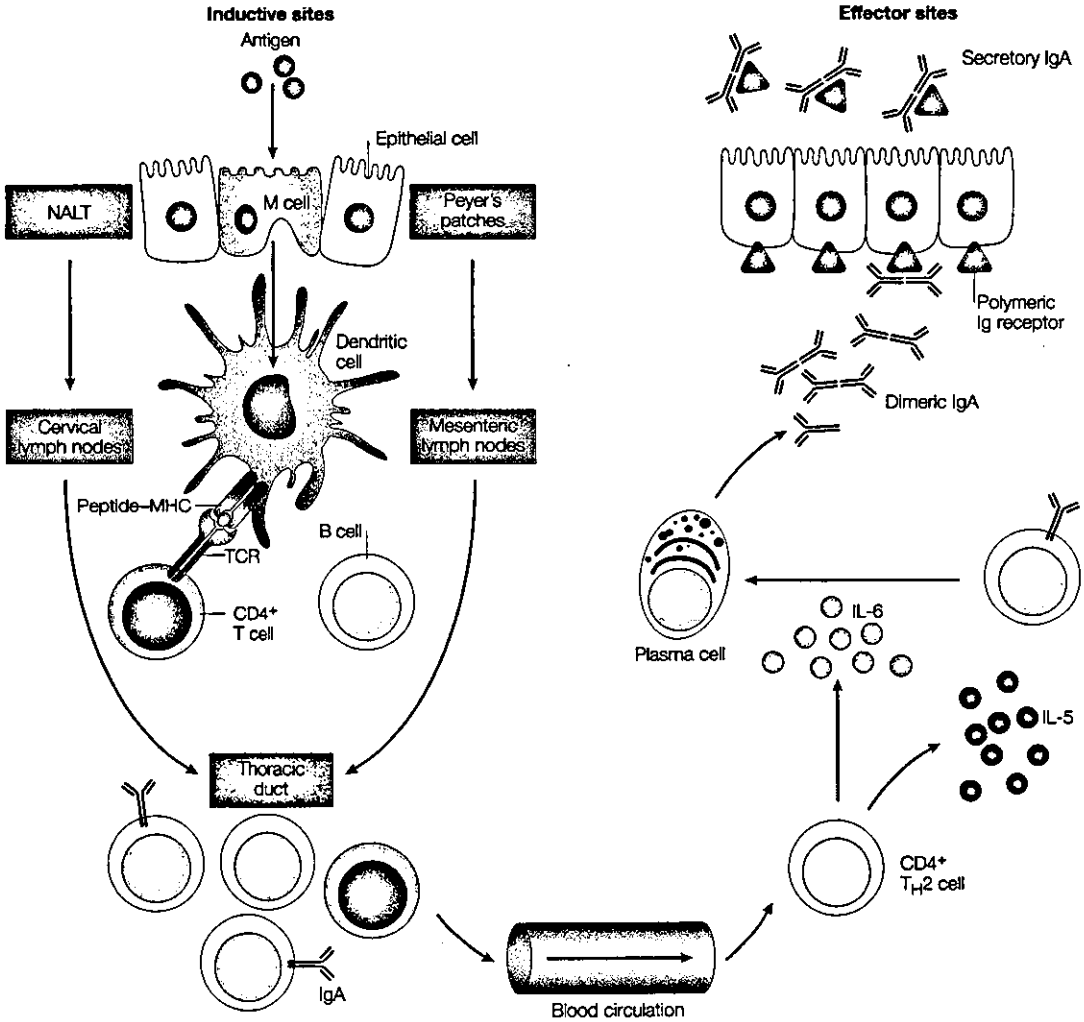

Figure 1 | **The common mucosal immune system.** Luminal antigens are transported to the nasopharynx-associated lymphoid tissue (NALT) and Peyer's patches through microfold (M) cells that are present in the epithelium overlying NALT and Peyer's-patch follicles. Dendritic cells process and present antigens to T cells in these lymphoid tissues. CD4$^+$ T cells that are stimulated by dendritic cells then preferentially induce IgA-committed B-cell development in the germinal centre of the lymphoid follicle. After IgA class switching and affinity maturation, B cells rapidly migrate from NALT and Peyer's patches to the regional cervical lymph nodes and mesenteric lymph nodes respectively, through the efferent lymphatics. Finally, antigen-specific CD4$^+$ T cells and IgA$^+$ B cells migrate to effector sites (such as the nasal passage and intestinal lamina propria) through the thoracic duct and blood circulation. IgA$^+$ B cells and plasmablasts then differentiate into IgA-producing plasma cells in the presence of cytokines (such as interleukin-5 (IL-5) and IL-6) that are produced by T helper 2 (T_H2) cells, and they subsequently produce dimeric (or polymeric) forms of IgA. These dimeric forms of IgA then become secretory IgA by binding to polymeric Ig receptors (which become the secretory component in the process of secretory IgA formation) that are displayed on the monolayer of epithelial cells lining the mucosa. Secretory IgA is then released into the nasal passage and intestinal tract. TCR, T-cell receptor.

COMMON MUCOSAL IMMUNE SYSTEM (CMIS). An integrated pathway that allows communication between the organized mucosa-associated lymphoid tissues (inductive sites) and the diffuse mucosal tissues (effector sites), enabling the induction and regulation of host-protective immunity against pathogenic microorganisms.

HIGH ENDOTHELIAL VENULES (HEVs). Venules (small veins that join capillaries to larger veins) that have a high-walled endothelium and are present in the paracortex of lymph nodes and tonsils, as well as in the interfollicular areas of Peyer's patches. HEVs are essential for lymphocyte homing to secondary lymphoid organs.

NASOPHARYNX-ASSOCIATED LYMPHOID-TISSUE (NALT) ANLAGEN
The site for the initiation of NALT development. At this site, the accumulation of $CD3^-CD4^+CD45^+$ cells and the expression of peripheral-node addressin (PNAD) by venules are observed in infant nasal tissues.

we have observed the formation of NALT in adult mice that were born and raised under germ-free conditions (H.K. and S.F., unpublished observations). Nonetheless, it is a strong possibility that initiation of NALT genesis is programmed to be activated after birth, and the subsequent maturation process is controlled by environmental antigens.

Contribution of cytokines to Peyer's-patch and lymph-node organogenesis. To show that cytokine-mediated NALT organogenesis is unique, it is important to summarize the mechanisms of Peyer's-patch and lymph-node organogenesis for comparative purposes. A family of pro-inflammatory cytokines that consists of lymphotoxin (LT) and tumour-necrosis factor (TNF), and their corresponding receptors (LT-β receptor (LT-βR), TNF receptor p55 (TNFRp55) and TNFRp75), creates a condition of 'programmed inflammation', which controls secondary lymphoid-tissue genesis[8,9] (TABLE 1). LTs are essential for secondary lymphoid-tissue organogenesis that is associated with the mucosal immune system, because deletion of either the genes that encode LT or the LT receptors, or artificial blockade of the interaction between the cytokine and its receptor during the embryonic period, results in the inhibition of both Peyer's-patch and peripheral lymph-node development[8,10,11]. For example, deletion of the *Lt-α* gene prevented Peyer's-patch formation and greatly limited the number of lymph nodes that developed[8]. LT-α forms LT-$\alpha_1\beta_2$ heterotrimers that can transduce an activation signal through the LT-βR, contributing to the organization of secondary lymphoid tissues[10]. When an LT-βR–Ig fusion protein was infused to antagonize the biological function of the LT-$\alpha_1\beta_2$ heterotrimer, lymphoid tissue formed at different anatomical locations depending on which embryonic stage was perturbed by introduction of the fusion protein[10]. This finding shows that the timing of secondary lymphoid-tissue development is regulated during embryogenesis[10,12]. We also found that the infusion of LT-βR–Ig between embryonic day (E) 15 and E17 suppressed Peyer's-patch development but had no effect on the formation of lymph nodes[13]. These studies clearly indicate the importance of the programmed inflammation that is mediated by LT-$\alpha_1\beta_2$ and the LT-βR for the genesis of Peyer's patches (TABLE 1; FIG. 3), but it is also known that another membrane-bound member of the TNF family, LIGHT, can bind to the LT-βR[14]. However, lymph nodes and Peyer's patches develop in the absence of LIGHT[15]. These findings indicate that the LT-$\alpha_1\beta_2$–LT-βR interaction is the essential component of programmed inflammation that initiates Peyer's-patch genesis at a particular time during the gestational period.

An additional cytokine that is associated with the mucosal immune system, namely interleukin-7 (IL-7), also has a crucial role in the initiation of Peyer's-patch genesis. IL-7 is produced by both mouse and human intestinal epithelial cells[16,17], and it provides stimulation and growth signals for neighbouring intestinal intraepithelial γδ T CELLS[16,18]. In mice that are deficient in the IL-7 receptor α-chain (*Il-7rα*$^{-/-}$), only the formation of Peyer's patches, and not lymph nodes, was impaired[19]. Similarly, when IL-7Rα function was blocked by administration of a single injection of an antagonistic monoclonal antibody to pregnant mothers on E15.5, the resulting offspring were deficient in Peyer's patches but showed normal lymph-node development[12]. These findings further emphasize that the LT-βR- and IL-7R-mediated tissue-genesis programme is crucial for the initiation of Peyer's-patch formation at the appropriate stage of embryogenesis (E14–E17) (FIGS 2,3).

Recently, a model that describes the development of Peyer's patches was proposed on the basis of this evidence. It was shown that lymphoid-lineage

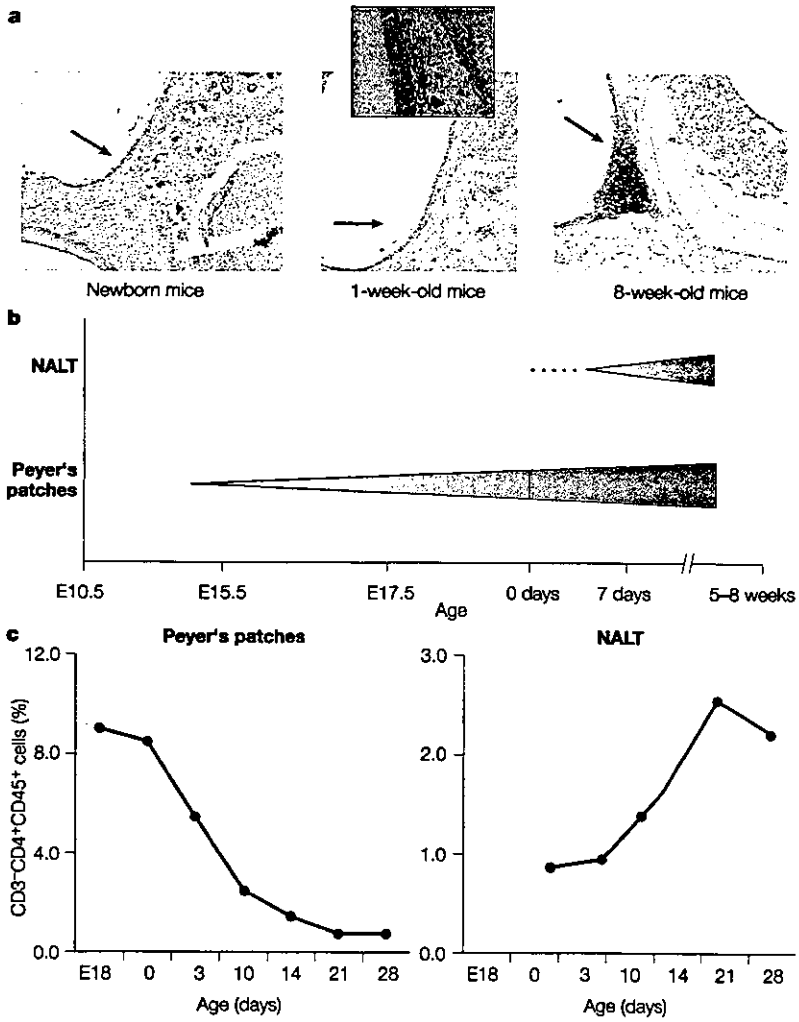

Figure 2 | **Chronological differences between NALT- and Peyer's-patch tissue genesis.** **a** | Nasal tissue from newborn mice (day 0) is characterized by an absence of peripheral-node addressin (PNAD)-expressing high endothelial venules (HEVs). The NASOPHARYNX-ASSOCIATED LYMPHOID-TISSUE (NALT) ANLAGEN from one-week-old mice shows a small accumulation of lymphoid cells around a single PNAD-expressing HEV in the nasal tissue. In eight-week-old mice, NALT contains numerous PNAD-expressing HEVs. This figure is reproduced with permission from REF. 5 © Elsevier (2002). **b** | The formation of NALT therefore starts after birth, whereas the development of Peyer's patches is initiated during embryogenesis. **c** | These kinetic differences in the initiation of tissue genesis of NALT and Peyer's patches are also supported by the appearance and frequency of $CD3^-CD4^+CD45^+$ inducer cells in nasal and intestinal tissues. The inducer cells accumulate postnatally at the site of NALT formation, whereas high numbers of these cells are observed in Peyer's patches during the gestational period. E, embryonic day.

Table 1 | **Unique organogenesis of NALT characterized by study of gene-manipulated mice**

Mice	NALT	Lymph nodes	Peyer's patches	References
Il-7rα$^{-/-}$	Disorganized	+	–	5,19,31
Lt-α$^{-/-}$	Disorganized	–	–	5,8,31
Lt-β$^{-/-}$	Disorganized	CLN and MLN	–	5,11
Lt-βr$^{-/-}$	ND	–	–	9
LT-βR–Ig	+	+/–	–	5,10
aly/aly (*Nik*$^{-/-}$)	Disorganized	–	–	5,27,28
Id2$^{-/-}$	–	–	–	5,30
Ror-γ$^{-/-}$	+	–	–	29,31,33
Trance$^{-/-}$	Disorganized	–	+	31
Cxcr5$^{-/-}$*Cxcl13*$^{-/-}$	ND	CLN and MLN	Reduced number	21,24

aly/aly, alymphoplasia mouse; CLN, cervical lymph node; *Cxcl13*, CXC-chemokine ligand 13; *Cxcr5*, CXC-chemokine receptor 5 (receptor for Cxcl13); *Id2*, inhibitor of DNA binding 2; *Il-7r*, interleukin-7 receptor; *Lt*, lymphotoxin; *Lt-βr*, Lt-β receptor; LT-βR–Ig, lymphotoxin-β-receptor–Ig fusion protein; MLN, mesenteric lymph node; NALT, nasopharynx-associated lymphoid tissue; ND, not determined; *Nik*, nuclear-factor-κB-inducing kinase; *Ror-γ*, retinoic-acid-receptor-related orphan receptor-γ; *Trance*, tumour-necrosis-factor-related activation-induced cytokine.

IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ cells that are considered to be PEYER'S-PATCH INDUCERS express CXC-chemokine receptor 5 (CXCR5) and can produce membrane-associated LT-$\alpha_1\beta_2$ heterotrimer, whereas mesenchymal-lineage VCAM1$^+$ and intercellular adhesion molecule 1 (ICAM1)$^+$ PEYER'S-PATCH ORGANIZERS express the LT-βR[20,21] (FIG. 3). Following stimulatory signals that are provided through the IL-7R, Peyer's-patch inducers express LT-$\alpha_1\beta_2$, which activates Peyer's-patch organizers through the LT-βR; and in turn, Peyer's-patch organizers produce chemokines, such as CXC-chemokine ligand 13 (CXCL13) and CC-chemokine ligand 19 (CCL19), which stimulate Peyer's-patch inducers through CXCR5 and CC-chemokine receptor 7 (CCR7) (REF. 22). The reciprocal interaction between inducer and organizer cells through chemokine and cytokine receptors is essential for the formation of Peyer's patches (FIG. 3), and the loss of any component of either of the signalling programmes is sufficient to disrupt secondary lymphoid-tissue development, as indicated by the loss of Peyer's patches in LT-βR-deficient and IL-7Rα-deficient mice[9,23]. Furthermore, deletion of the gene that encodes CXCR5 partially reduces the formation and number of Peyer's patches[24] (TABLE 1). The lack of Peyer's patches and lymph nodes in alymphoplasia (*aly/aly*) mice, which have defective NIK (nuclear factor-κB (NF-κB)-inducing kinase) function, also fits this model, because recent analyses have established that NIK is essential for the transduction of signals through the TNFR family, including those through the LT-βR[25,26]. So, *aly/aly* mice lack Peyer's patches because the NIK mutation inhibits the reciprocal interaction between Peyer's-patch inducers and organizers that is mediated through LT-$\alpha_1\beta_2$ and the LT-βR[27,28]. Further evidence in support of this model comes from studies showing that mice that lack the CD3$^-$CD4$^+$CD45$^+$ inducer cells, owing to genetic deletion of the transcriptional regulators ID2 (inhibitor of DNA binding 2) or ROR-γ (retinoic-acid-receptor-related orphan receptor-γ), also lack Peyer's patches and lymph nodes[29,30].

LT-βR- and IL-7R-independent NALT organogenesis. Because Peyer's-patch formation requires a cytokine-signalling cascade that involves the IL-7R and the LT-βR (TABLE 1; FIG. 3), we examined whether an identical receptor-signalling cascade would trigger NALT development. The formation of NALT was studied in mice lacking Peyer's patches and/or lymph nodes, including *Lt-α*$^{-/-}$, *Lt-β*$^{-/-}$ and *aly/aly* mice, and mice that were treated *in utero* with the LT-βR–Ig fusion protein[5] (TABLE 1). Nasal lymphoid tissue was detected in all mouse strains lacking Peyer's patches or both Peyer's patches and lymph nodes because of a deficiency in the LT-βR-mediated pro-inflammatory cytokine cascade[5]. A separate study by Harmsen and colleagues[31] confirmed the formation of NALT in the absence of LT-βR-mediated signalling. The authors also showed that NALT formation was reconstituted in mice that were deficient in both TNF and LT-α by the adoptive transfer of wild-type bone marrow, even though Peyer's patches did not develop in these mice[31]. These findings further support the idea that NALT development does not conform with the model of programmed inflammation that is required for the genesis of Peyer's patches (FIG. 3).

Because Peyer's-patch formation has also been shown to require the IL-7R-mediated signalling pathway, in addition to the LT-βR cascade, NALT development was examined in IL-7R-deficient mice. NALT, but not Peyer's patches, was found to develop in IL-7R-deficient mice[5,31]. Taken together, these findings directly show that NALT formation is independent of IL-7R- and LT-βR-mediated tissue genesis (FIG. 3).

CD3$^-$CD4$^+$CD45$^+$ cells in NALT organogenesis. A unique subset of mononuclear cells that are characterized as being CD3$^-$CD4$^+$CD45$^+$ have been shown to function as inducer cells for the organogenesis of secondary lymphoid tissues, including Peyer's patches[21]. So, a high frequency of CD3$^-$CD4$^+$CD45$^+$ cells is observed in the intestinal tract at embryonic stages of development (FIG. 2). Furthermore, *Id2* has been identified as one of

γδ T CELLS
T cells that express heterodimers consisting of the γ- and δ-chains of the T-cell receptor. They are present mainly in the intestinal epithelium as intraepithelial lymphocytes (IELs). Although the exact function of γδ T cells (or IELs) is still unknown, it has been suggested that mucosal γδ T cells are involved in the innate immune responses of the mucosal immune system.

PEYER'S-PATCH INDUCERS
CD3$^-$CD4$^+$CD45$^+$ cells that express the interleukin-7 receptor and lymphotoxin-$\alpha_1\beta_2$. They differentiate from fetal liver cells and can induce Peyer's-patch formation during the embryonic stage.

PEYER'S-PATCH ORGANIZERS
Lymphotoxin-β-receptor-positive stromal cells that are present in the anlagen of Peyer's patches and also express both VCAM1 (vascular cell-adhesion molecule 1) and ICAM1 (intercellular adhesion molecule 1). Peyer's-patch development is initiated with the cooperation of Peyer's-patch inducers.

the genes that is responsible for the induction of these $CD3^-CD4^+CD45^+$ inducer cells[30]. Not surprisingly, deletion of the *Id2* gene completely impaired the genesis of all secondary lymphoid tissues, including both NALT and Peyer's patches[5,30]. $CD3^-CD4^+CD45^+$ inducer cells were shown to accumulate at the site of NALT formation after birth[5] (FIG. 2), thereby clarifying the role of these cells in the induction of NALT development. To directly show that $CD3^-CD4^+CD45^+$ cells are responsible for the genesis of NALT, fetal liver cells were adoptively transferred from wild-type $Id2^{+/+}$ mice to newborn $Id2^{-/-}$ mice. Seven days after this transfer, $CD3^-CD4^+$ cells were observed to have migrated to the site of NALT formation, and 7 weeks after transfer, a NALT-like structure was detected[5]. These findings are the first to show directly *in vivo* that $CD3^-CD4^+CD45^+$ inducer cells are essential for the initiation of organogenesis of secondary lymphoid tissues (such as NALT).

The transcriptional regulator ROR-γ has also been shown to be required for the development of $CD3^-CD4^+CD45^+$ inducer cells[29,32]. Deletion of the gene that encodes ROR-γ suppressed Peyer's-patch and lymph-node organogenesis[29,33]. However, NALT development has been reported in ROR-γ-deficient mice[31]. This might indicate that although NALT and Peyer's patches have inducer cells of the same phenotype — that is, $CD3^-CD4^+CD45^+$ — those inducer cells can be classified into two distinct groups on the basis of their dependence on ROR-γ and ID2 (FIG. 4). We think that a population of IL-7R-expressing $CD3^-CD4^+CD45^+$ inducer cells that are essential for Peyer's-patch tissue genesis are regulated by both ROR-γ and ID2, whereas a subset of inducer cells that lack IL-7R expression and are required for NALT genesis are regulated by ID2 but not ROR-γ (FIG. 4) — although this has not been proven experimentally. So, the two $CD3^-CD4^+CD45^+$ inducer-cell populations for NALT and Peyer's-patch organogenesis might be determined or programmed at the level of the transcriptional regulator ROR-γ (FIG. 4). In addition, it is also possible that a population of $CD3^-CD4^+CD45^+$ inducer cells is absent in both $Id2^{-/-}$ mice and $Ror\text{-}\gamma^{-/-}$ mice, and instead, another as-yet-undefined cell population — which can substitute for the classical inducer cells during the formation of NALT — is present in $Ror\text{-}\gamma^{-/-}$ mice but not $Id2^{-/-}$ mice. Further studies are required to investigate these possibilities and others.

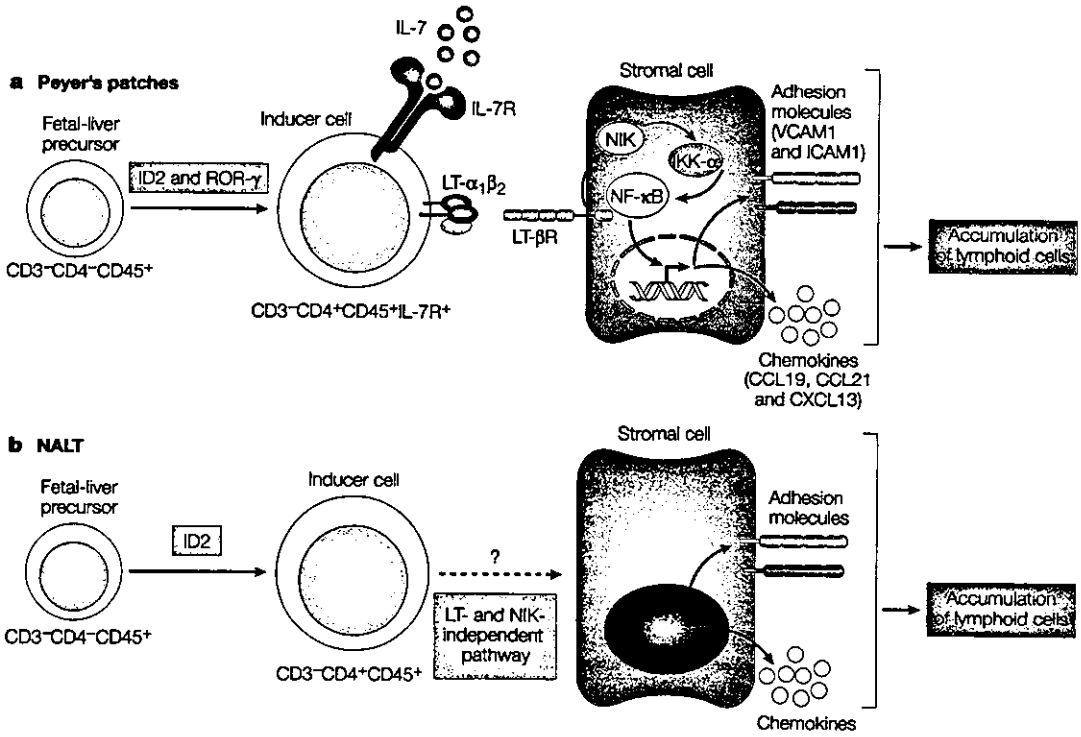

Figure 3 | **Comparison of the organogenesis programme of NALT and Peyer's patches.** $CD3^-CD4^+CD45^+$ cells are considered to be the common inducers of secondary lymphoid tissue. ID2 (inhibitor of DNA binding 2) is indispensable for the induction and differentiation of these inducer cells from their fetal-liver precursors (which have the phenotype $CD3^-CD4^-CD45^+$). **a** | For Peyer's patches, after activation through the interleukin-7 receptor (IL-7R) or TRANCE (tumour-necrosis-factor-related activation-induced cytokine), these $CD3^-CD4^+CD45^+$ cells express the lymphotoxin-$\alpha_1\beta_2$ (LT-$\alpha_1\beta_2$) heterotrimer, which then binds to the LT-β receptor (LT-βR) displayed on stromal cells and induces signal transduction through NIK (nuclear factor-κB (NF-κB)-inducing kinase). In turn, NIK promotes the expression of adhesion molecules and/or chemokines. These homing molecules trigger the accumulation of lymphoid cells at the site of Peyer's patches. So, the IL-7R- and LT-βR-mediated signals are essential for the tissue genesis of Peyer's patches. **b** | The development of $CD3^-CD4^+CD45^+$ cells in nasopharynx-associated lymphoid tissue (NALT) also requires ID2; however, the initiation of NALT organogenesis is independent of signalling that involves the IL-7R, LT-$\alpha_1\beta_2$–LT-βR interactions and NIK. CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; ICAM1, intercellular adhesion molecule 1; IKK-α, inhibitor of NF-κB (IκB) kinase-α; ROR-γ, retinoic-acid-receptor-related orphan receptor-γ; VCAM1, vascular cell-adhesion molecule 1.