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

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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

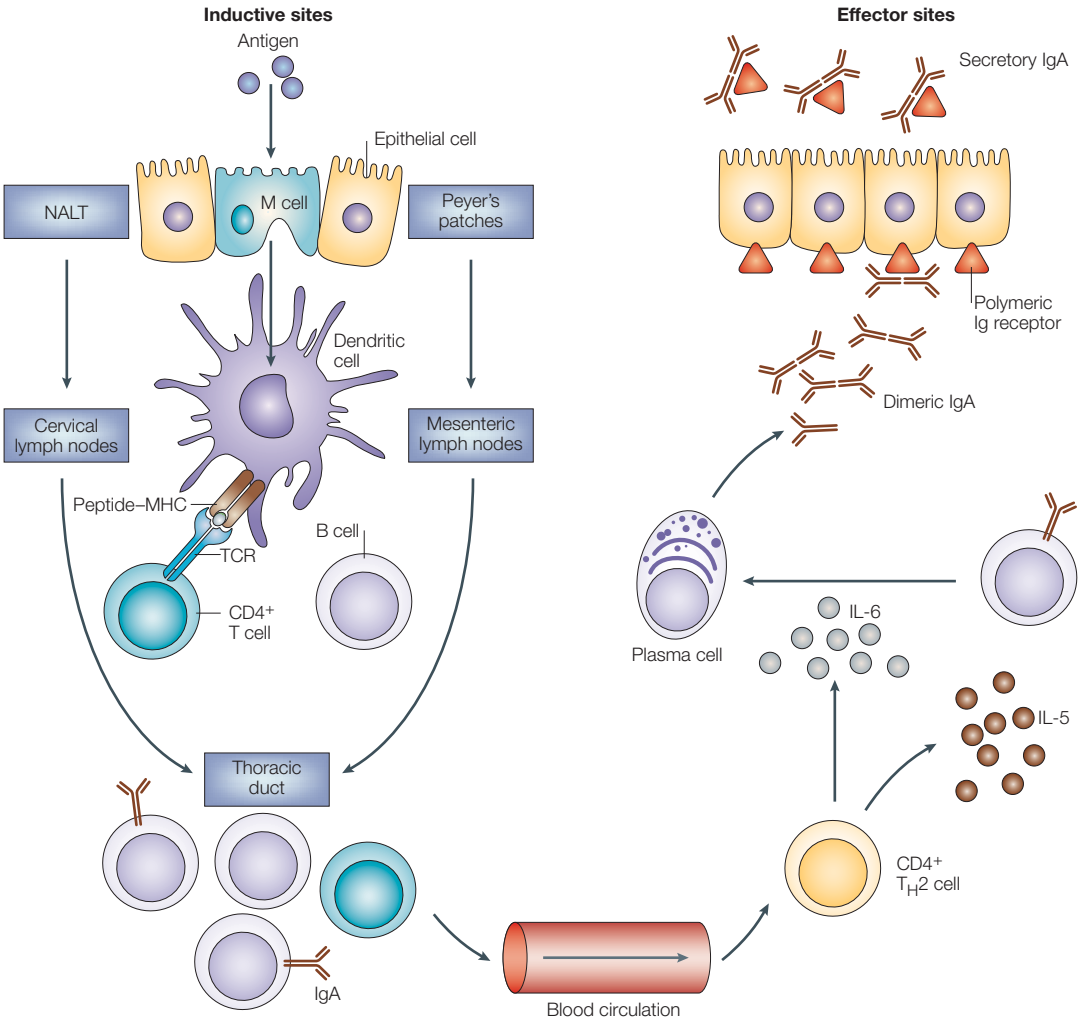

Figure 1 | **The common mucosal immune system.** Luminal antigens are transported to the nasopharynx-associated lymphoid tissue (NALT) and Peyer's patches through microfold (M) cells that are present in the epithelium overlying NALT and Peyer's-patch follicles. Dendritic cells process and present antigens 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

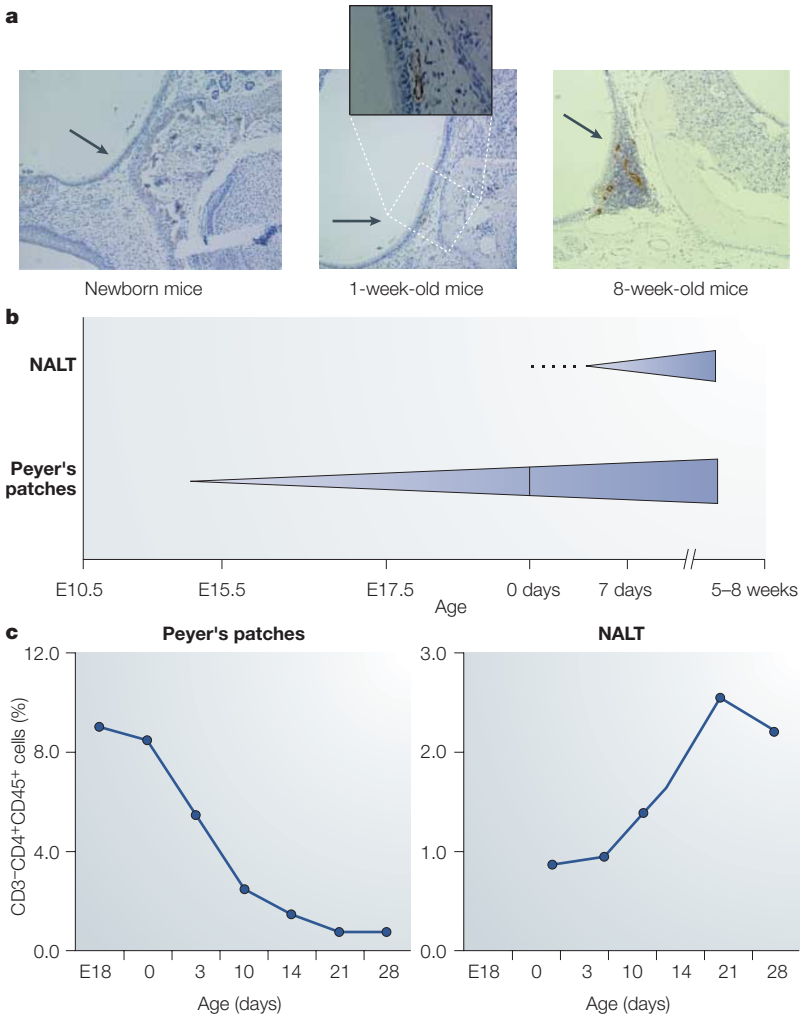

Figure 2 | **Chronological differences between NALT- and Peyer's-patch tissue genesis.** **a** | Nasal tissue from newborn mice (day 0) is characterized by an absence of peripheral-node addressin (PNAD)-expressing high endothelial venules (HEVs). The NASOPHARYNX-ASSOCIATED LYMPHOID-TISSUE (NALT) ANLAGEN 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.

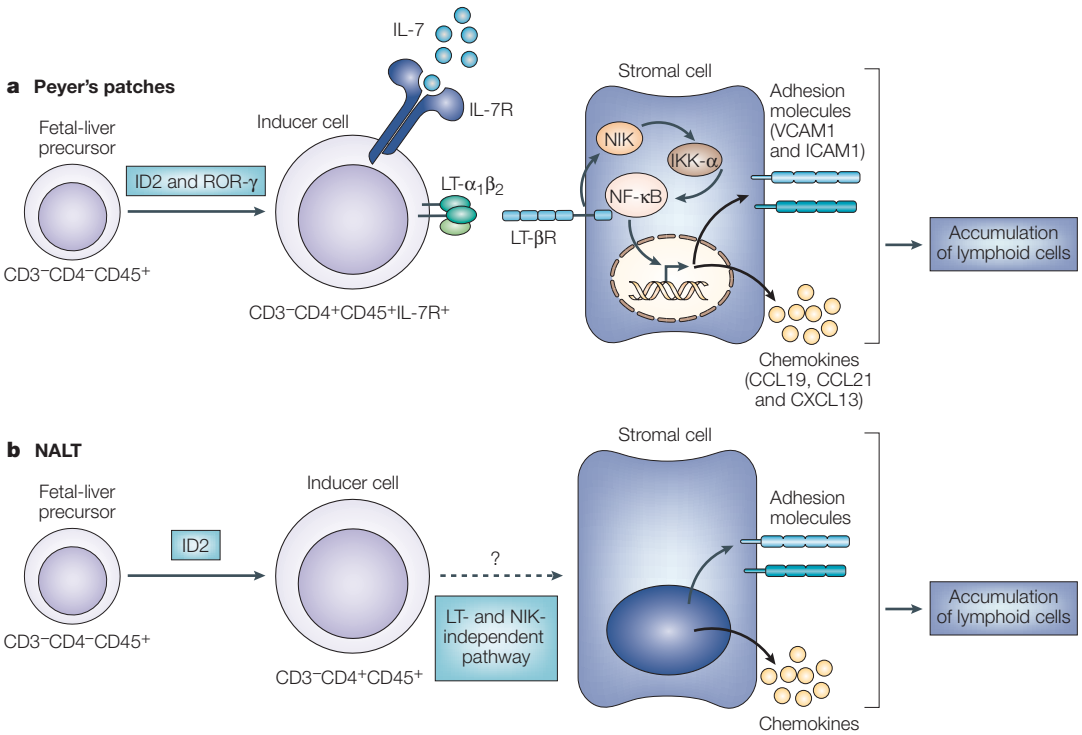

Figure 3 | **Comparison of the organogenesis programme of NALT and Peyer's patches.** $CD3^-CD4^+CD45^+$ cells are considered to be the common inducers of secondary lymphoid tissue. ID2 (inhibitor of DNA binding 2) is indispensable for the induction and differentiation of these inducer cells from their 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.

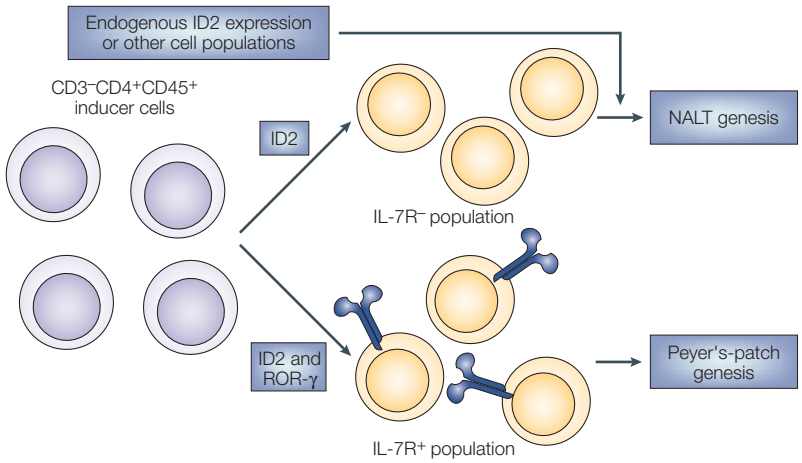

Figure 4 | **Model for the induction of organogenesis of NALT and Peyer's patches by two subsets of CD3⁻CD4⁺CD45⁺ inducer cells.** CD3⁻CD4⁺CD45⁺ cells differentiate from fetal-liver progenitors. We propose that both ROR-γ (retinoic-acid-receptor-related orphan receptor-γ) and ID2 (inhibitor of DNA binding 2) are essential for the generation of interleukin-7 receptor (IL-7R)-expressing CD3⁻CD4⁺CD45⁺ cells for the induction of Peyer's-patch organogenesis. By contrast, the generation of the IL-7R⁻CD3⁻CD4⁺CD45⁺ inducer cells that are involved in nasopharynx-associated lymphoid tissue (NALT) organogenesis is regulated by ID2 but not ROR-γ. However, this model remains to be tested experimentally, and other possibilities exist (see main text). NALT genesis in ID2-deficient mice can be initiated by the adoptive transfer of CD3⁻CD4⁺CD45⁺ cells from wild-type mice, but the maturation of NALT formation is incomplete. This indicates that other cell populations, or the endogenous expression of ID2 at the site of NALT, might be required for the full maturation of NALT.

WALDEYER'S RING
Human nasopharynx-associated lymphoid tissues, including the palatine tonsils and adenoids, which are considered to have an important role in the induction and modulation of mucosal immunity in the upper respiratory tract.

MIDDLE CONCHA
Bony plate that extends from the central section of the lateral wall of the nasal cavity.

T_H0 CELLS
Precursors of T helper 1 (T_H1) cells and T_H2 cells, which produce both interferon-γ and interleukin-4. This T-cell population has the capacity to become T_H1- and/or T_H2 cells.

CLASS-SWITCH RECOMBINATION
Molecular alteration of the constant-region gene of the immunoglobulin heavy chain (C_H) that leads to a switch in expression from the Cμ (or Cδ) region to one of the other C_H genes. This leads to a switch in the class of the immunoglobulin that is displayed on the cell-surface of the B cell (and that subsequently differentiating plasma cells produce) — from IgM (or IgD) to IgG, IgA or IgE — without altering the specificity of the immunoglobulin.

Immunological features of NALT

In rodents, NALT is found on both sides of the nasopharyngeal duct, dorsal to the cartilaginous soft palate, and it is considered analogous to WALDEYER'S RING in humans[34,35]. Also, in a recent study, a NALT-like structure of lymphocyte aggregates that form follicles was identified in human nasal mucosa, particularly in the MIDDLE CONCHA of children less than two years of age[36], indicating that an equivalent to mouse NALT can develop in humans. NALT consists of follicle-associated epithelium (FAE), HEVs, and T-cell- and B-cell-enriched areas. Antigen-sampling M cells are present in the epithelium of NALT, which is specialized for antigen uptake similar to the FAE of Peyer's patches[7,37]. Antigen-presenting cells, including dendritic cells (DCs) and macrophages, are also found in NALT[38]. So, NALT contains all of the lymphoid cells that are required for the induction and regulation of mucosal immune responses to antigens that are delivered to the nasal cavity. For example, the intranasal administration of reovirus resulted in the formation of germinal centres in NALT, leading to the clonal expansion of antigen-induced IgA⁺ B cells and the subsequent generation of reovirus-specific IgA in the respiratory and intestinal tracts[39]. Moreover, reovirus-specific CTLs were also induced in NALT with a high frequency. These findings show that NALT can be a potent inductive site for the mucosal immune system. In addition to the induction of positive immune responses, the nasal deposition of antigen has been shown to be effective for the induction of systemic unresponsiveness — a form of mucosally induced tolerance[40]. So, NALT has been shown to be involved in the generation of positive- and negative-regulatory signals for the induction of antigen-specific immunity and tolerance respectively. The cellular and molecular contributions of the immunocompetent cells present in NALT to the generation of tolerance to mucosally exposed antigens are unknown. Because little is known about the induction of nasally induced tolerance, we focus here on the role of NALT in the induction of protective immunity.

T_H0 environment. Characterization of the mRNA that encodes T_H1 and T_H2 cytokines in CD4⁺ T cells isolated from mouse NALT revealed a dominant cytokine profile of T_H0 CELLS, indicating that these T cells are capable of becoming T_H1 or T_H2 cells immediately after antigen exposure of the nasal tract[41–43]. CD4⁺ T cells isolated from NALT of naive wild-type mice are T_H0 cells[42], so they can become either T_H1 or T_H2 cells depending on the identity of the nasally administered antigen. Nasal delivery of protein antigens (such as bacterial cell-wall components or virus-associated antigens) together with cholera toxin as a mucosal adjuvant induces antigen-specific T_H2-type responses that promote the generation of antigen-specific IgA-producing B cells, both in the nasal passages and at distant mucosal effector sites, including the genito-urinary, respiratory and intestinal tracts[41,44,45]. By contrast, intranasal vaccination with antigen-expressing recombinant *Mycobacterium bovis* bacillus Calmette-Guérin (rBCG) results in T_H1-cell-mediated immunity[43].

IgA class switching. Peyer's patches have long been thought to be the sites for the initiation of CLASS-SWITCH RECOMBINATION (CSR) of μ- to α-gene expression in the gastrointestinal tract, because they contain all of the cellular and microarchitectural elements that are required for the generation of IgA-committed B cells, including germinal-centre-containing B-cell follicles, a FOLLICULAR DC network and an interfollicular T-cell area[1,46,47]. The germinal-centre region contains a high frequency of IgM⁺B220⁺ B cells that express activation-induced cytidine deaminase (AID), which is essential for μ-to α-gene conversion[48]. In an early study, it was shown that incubation of IgM⁺IgA⁻ B cells isolated from Peyer's patches in the presence of the cytokine transforming growth factor-β (TGF-β) resulted in the generation of IgM⁻IgA⁺ B cells[49–51]. These post-switch IgA-committed B cells then migrated to mucosal effector tissues (such as the intestinal lamina propria), a process mediated by a group of homing and chemokine receptors and their ligands (such as MADCAM1–$\alpha_4\beta_7$-integrin and CCL25–CCR9 interactions)[52,53] (discussed later). In the intestinal lamina propria, these cells became IgA⁺ plasma cells in the presence of the IgA-enhancing cytokines IL-5, IL-6 and IL-10 (REFS 50,54–58). So, it was generally accepted that organized lymphoid structures of MALT, such as Peyer's patches, function as the inductive sites for generating IgA-committed B cells through μ- to α-gene CSR, whereas the diffuse tissues of the intestinal lamina propria function as effector sites for the production of IgA[1,2] (FIG. 1). However, the finding that IgA class switching can occur in the intestinal lamina propria without involvement of

the Peyer's patches[59] cast these assumptions into doubt. Stromal-cell-derived TGF-β present in the intestinal lamina propria was shown to trigger IgM^+B220^+ cells to switch to IgA^+ B cells[59]. Mice with a deficiency in the programmed-inflammation-associated cytokine LT-α do not form Peyer's patches, so the levels of IgA responses are reduced compared with those of wild-type mice[60]. However, reconstitution of *Lt-α*$^{-/-}$ mice with LT-expressing bone-marrow cells, or transplantation of an intestinal segment from recombination-activating gene (*Rag*)$^{-/-}$ mice to *Lt-α*$^{-/-}$ mice, resulted in the recovery of IgA responses[60]. These findings imply that at least some IgA-committed B cells can develop, even in the absence of Peyer's patches. *Lt-α*$^{-/-}$ mice have also been shown to be capable of inducing antigen-specific IgA responses to orally administered *Salmonella typhimurium*, despite the absence of organized lymphoid tissues associated with the mucosal compartment[61]. Although these findings provide supporting evidence that the environment of the diffuse lamina propria region is self-sufficient for the μ- to α-gene CSR that leads to the generation of IgA-committed B cells, it is also possible that unidentified, programmed-inflammation-independent miniature lymphoid aggregates, and recently characterized isolated lymphoid follicles[62], have a role in mounting IgA responses in cases of Peyer's-patch deficiency.

The finding that IgA-specific CSR can occur in diffuse mucosal effector tissues indicates that organized mucosal tissue is not essential for the generation of IgA-committed B cells in the digestive tract[59], although this new view is still controversial[63,64]. Because it has been shown that intestinal IgA is produced by two groups of cells, B1 cells and B2 CELLS[63,65,66], an interesting possibility is that IgA-specific CSR of B1 cells does not require organized lymphoid structures, whereas these structures are essential for IgA-isotype switching in B2 cells. In support of this view, we have shown that most B cells in gut-associated lymphoid tissues are B2 cells, whereas B1 cells are located preferentially in the intestinal lamina-propria region[66]. So, we think that the initial antigenic stimulation for the triggering of IgA-isotype switching in B2 cells might be provided by antigen sampling through M cells that are located in the dome epithelium (or FAE) of Peyer's patches. By contrast, the IgA-specific CSR process that occurs for B1 cells might be triggered by antigens sampled through newly identified villous M cells that are located adjacent to lamina-propria regions that do not contain observable lymphoid-like structures[67].

Because NALT is of similar importance for the initiation of IgA^+ B-cell responses as Peyer's patches, we have investigated whether IgA-isotype switching can also occur in the diffuse tissue of the nasal passage. IgM^+B220^+ B cells, which are a prerequisite for CSR, were found in the organized inductive sites (NALT) but were mostly absent from the diffuse effector tissues (nasal passage) of the respiratory mucosal immune system[68]. Similarly, IgM^+B220^+ B cells were observed in the organized Peyer's patches of the intestinal tract but not in the intestinal lamina propria. So, in this study, IgM^+B220^+ B cells that are preconditioned to undergo IgA class switching are selectively located in the organized mucosa-associated inductive tissues of NALT and Peyer's patches[68]. This finding was confirmed by the molecular analysis of IgA CSR-associated mRNA specific for AID, Iα-Cμ CIRCULAR TRANSCRIPTS and Iμ-Cα TRANSCRIPTS. Because the expression of AID and the Iα-Cμ circular transcript are upregulated preferentially during μ- to α-gene conversion and then quickly down-regulated, these molecular events are considered to be a hallmark of B cells that are undergoing IgA class switching[69]. The expression of Iμ-Cα transcripts indicates the completion of IgA-specific CSR[59]. This analysis showed that the expression of AID-, Iα-Cμ circular transcript- and Iμ-Cα transcript-specific mRNA was restricted to the organized mucosal inductive tissues of NALT and Peyer's patches but was not found in the diffuse effector tissues of the nasal passage and intestinal lamina propria[68]. Furthermore, these organized mucosal lymphoid tissues are known to be associated with B2 cells[66]. So, these findings indicate that the IgA class switching, at least for B2 cells, requires the organized lymphoid structures of NALT and Peyer's patches in the aero-digestive tract. NALT was also recently shown to be an important site for the generation of memory B cells, which produce high-affinity IgA[70]. Taken together, these findings show that NALT contains all of the immunocompetent cells that are required for the induction and regulation of antigen-specific T_H1- or T_H2-cell-mediated responses and B-cell immune responses.

Differences between NALT- and Peyer's-patch-initiated immune responses. NALT and Peyer's patches are thought to have similar immunological characteristics and biological functions, as well as to contain the same types of resident immunocompetent cell. So, similar to oral immunization, nasal immunization can stimulate antigen-specific T_H1- or T_H2-cell-mediated responses and IgA responses in distant mucosal effector tissues[1,2,41,43–45]. However, in general, NALT-targeted immunization effectively induces antigen-specific immunity in the respiratory and reproductive tissues, whereas Peyer's-patch-targeted immunization promotes the generation of protective immunity in the gastrointestinal-tract tissues[1,2]. Further support for a compartmentalized CMIS was provided recently when it was shown that nasal immunization induces the expression of high levels of CCR10 and $\alpha_4\beta_1$-integrin by IgA-committed B cells, allowing them to efficiently traffic to the respiratory and genito-urinary tracts, which express the corresponding ligands, CCL28 and VCAM1 (REFS 71,72). By contrast, orally induced IgA-committed B cells express CCR9 and CCR10, as well as $\alpha_4\beta_7$- and $\alpha_4\beta_1$-integrins, so they migrate to sites such as the small intestine, which express CCL25 and/or CCL28 together with MADCAM1 and/or VCAM1 (REF. 73).

So, despite NALT and Peyer's patches both belonging to the mucosal immune system, the subtle differences that we have discussed indicate that the tissue genesis and biological functions of NALT and Peyer's patches might differ because of their anatomically and

FOLLICULAR DC
(FDC). Cell with a dendritic morphology that is present in lymph nodes. These cells display on their surface intact antigens that are held in immune complexes, and B cells present in the lymph node can interact with these antigens. FDCs are of non-haematopoietic origin and are not related to dendritic cells.

B2 CELLS
$IgM^{low}IgD^{hi}MAC1^-B220^{hi}CD23^+$ cells that originate from bone marrow and are distributed to mucosal and systemic immune compartments for the continuous secretion of antibodies with high affinity and fine specificity.

Iα-Cμ CIRCULAR TRANSCRIPTS
Circular DNA molecules that are present in activated B cells and consist of Iα (intervening region-α) and Cμ genes. They are a hallmark of B cells that are in the process of IgA class switching.

Iμ-Cα TRANSCRIPTS
Germline transcripts that can be detected in IgA-committed B cells after class switching.

Table 2 | **Novel mucosal adjuvants and delivery systems for the development of nasal vaccines**

Antigen	Adjuvant and delivery vehicle	T_H cells	Secretory IgA	Serum IgG	Protective immunity	References
Influenza HA	mCTA–nLTB	$T_H2 > T_H1$	+	+	+	93
PspA	mCT	$T_H2 > T_H1$	+	+	+	90
V3J1	rBCG	$T_H1 > T_H0$	–	+	+	43
HIV gp160	HVJ liposome	$T_H1 = T_H2$	+	+	+	76

gp160, glycoprotein 160; HA, haemagglutinin; HVJ, haemagglutinating virus of Japan; nLTB, B subunit of native form of heat-labile enterotoxin; mCT, mutant cholera toxin; mCTA, A subunit of MCT; PspA, pneumococcal surface adhesin A; rBCG, recombinant *Mycobacterium bovis* bacillus Calmette-Guérin; T_H, T helper cell; V3J1, peptide containing neutralizing epitope of HIV.

environmentally distinct locations. Targeting the CMIS would therefore seem to be a logical choice for the development of a second generation mucosal (nasal or oral) vaccine to induce antigen-specific immune responses — such as a combination of T_H1-cell or T_H2-cell responses, CTL responses, and IgA and IgG responses — in the common and/or selective regions of the mucosal compartments, as well as at systemic sites, through the use of the NALT- and Peyer's-patch-initiated mucosal immune responses (FIG. 1).

NALT-based vaccine development

As we have described, NALT is one of the key components of the organized lymphoid tissue, and it contains all of the immunocompetent cells that are required for the induction of antigen-specific immune responses. It is therefore likely to have a central role in the development of a 'nasal vaccine'. Nasal vaccination has proven to be an effective regimen for the stimulation of the respiratory immune system[1,2,41,45]. Furthermore, this route of mucosal immunization can elicit both humoral and cell-mediated antigen-specific immune responses[1,2,41,43–45]. Another attractive feature of nasal immunization is that it requires a much smaller dose of antigen than does oral vaccination for the induction of antigen-specific mucosal and systemic immune responses, because the antigens are not exposed to degradation by digestive enzymes. Because intranasal administration of vaccine antigen alone has failed to fully stimulate NALT, researchers are attempting to develop an effective NALT-targeted vaccine-antigen delivery system and to develop a safe and effective immune-enhancing molecule (or adjuvant) for intranasal administration with the vaccine antigen.

NALT-targeted vaccine delivery. Antigens are known to be more immunogenic in particulate form than in soluble form, but they are vulnerable to antigen-degrading enzymes and acids that are associated with the mucosal environment. To overcome these obstacles, much effort has been focused on the creation of novel non-toxic and non-immunogenic vaccine vehicles that can effectively deliver even the soluble form of antigen to the organized mucosal inductive tissue. Such vehicles need to protect vaccine components from degradation, enhance their uptake from mucosal surfaces and perhaps function as an adjuvant. Among the various candidates for mucosal antigen delivery, Sendai-virus-associated fusion protein seems particularly suited to function as a molecule that guides antigen to the mucosal epithelium, because the Sendai virus itself uses this fusion protein for the invasion of respiratory epithelial cells[74]. A novel hybrid antigen-delivery vehicle has also been devised using this envelope fusion glycoprotein of Sendai virus (or using the haemagglutinating virus of Japan, HVJ); the fusion protein is displayed on the surface of liposomes (either fusogenic liposomes or HVJ liposomes) that contain the antigen of interest. When this delivery vehicle was used, intranasally administered antigen that was conjugated to green-fluorescent protein successfully reached the antigen-sampling M cells that are located in the epithelium of NALT[75]. Fusogenic liposomes were also found to effectively deliver antigen to epithelial cells and macrophages in both NALT and the nasal passages[75]. Furthermore, it was shown that an intranasally administered HVJ liposome containing the HIV glycoprotein 160 antigen (gp160; also known as env) was a powerful tool for inducing gp160-specific serum IgG, and gp160-specific mucosal IgA was also detected in nasal wash, saliva, faecal extract and vaginal wash[76] (TABLE 2). These findings show that the novel hybrid antigen-delivery vehicle of fusogenic liposomes (or HVJ liposomes) effectively transports vaccine antigen to NALT for the initiation of antigen-specific IgA responses at distant mucosal effector sites. Furthermore, this immunization method can also induce antigen-specific immune responses (such as production of IgG) in the systemic compartment (TABLE 2).

Because antigen-sampling M cells are scattered throughout the NALT epithelium[77], it seems logical to develop an M-cell-targeted nasal vaccine. One promising approach has been to use a molecule that is involved in the normal course of invasion of an infectious agent. Reovirus, an enteric pathogen, is known to invade its host through M cells that are located in the epithelium of Peyer's patches[78]. The 45-kDa viral haemagglutinin σ1 protein of reovirus has a crucial role in its attachment to and entry into M cells[79]. The virus has been shown to recognize mouse M cells that are present in the airways[80], and the recombinant form of the σ1 protein can bind to M cells that are associated with NALT epithelium[80]. On the basis of these findings, attempts have been made to develop an M-cell-targeted DNA vaccine using the σ1 protein as a guiding molecule[81]. When conjugated to a eukaryotic expression vector that encodes luciferase (known as pCMVLuc) and administered intranasally, the σ1 protein can specifically bind to the apical surface of M cells that are situated in the follicular epithelium

of NALT; it then leads to the generation of luciferase-specific serum IgG and mucosal IgA responses[80]. A nasal vaccine assembled using the σ1 protein and gp160 resulted in gp160-specific CTL responses in various mucosa-associated and systemic immune compartments, including reproductive tissue and spleen respectively[81]. These findings further emphasize the efficacy of NALT-targeted immunization for the induction of humoral and/or cell-mediated antigen-specific immune responses in mucosal and systemic immune compartments.

Because it only infrequently causes serious complications, BCG, a commonly used vaccine for the control of tuberculosis, is considered to be a low-risk vaccine. The recombinant form of BCG is a useful vaccine-antigen delivery vehicle, because it has strong adjuvant activity that can induce both humoral and cell-mediated immune responses[82]. Indeed, systemic administration of rBCG that expresses HIV antigen has been shown to effectively induce cell-mediated immunity[43,83]. Our own studies have shown that intransal administration of rBCG that expresses V3J1, a neutralizing epitope of HIV, can induce V3-peptide-specific IgG that has neutralizing activity for more than 0.5–1 years in both normal and immunodeficient (interferon-γ-deficient or *Il-4*$^{-/-}$) mice[43] (TABLE 2). Furthermore, V3J1–rBCG-induced serum IgG has also been shown to effectively neutralize a homologous strain of HIV[43]. Accordingly, rBCG shows promise as an effective nasal-immunization vehicle for the induction of prolonged antigen-specific antibody responses.

Creation of safe toxin-based adjuvants. Both cholera toxin that is produced by *Vibrio cholerae* and the heat-labile enterotoxin of *Escherichia coli* function as adjuvants to enhance mucosal and serum antibody responses to co-administered protein antigens delivered by oral or nasal routes[2,84]. Unfortunately, despite their efficacy as mucosal adjuvants, the native forms of cholera toxin (nCT) and heat-labile enterotoxin (nLT) cause severe diarrhoea and so are unsuitable for use in humans. To overcome these hurdles, researchers have substituted a single amino acid to generate non-toxic mutant forms of cholera toxin (mCT) and heat-labile enterotoxin (mLT)[85–88]; these retain the adjuvanticity of the native forms but do not induce the ribosylation of ADP that is associated with toxic activity. Our efforts to devise a safe first generation toxin-based adjuvant have focused on mCT S61F (in which phenylalanine replaces serine at position 61) and mCT E112K (in which lysine replaces glutamic acid at position 112); these mutations were created by making a single amino-acid substitution in the active centre of the ADP-ribosyltransferase in the A subunit of cholera toxin[89]. The two mutant forms of cholera toxin have been shown to be safe by *in vitro* analyses of ADP-ribosylation activity and cyclic AMP formation, as well as by *in vivo* examination for diarrhoea-like symptoms. When pneumococcal surface-protein A (PspA) — a new candidate vaccine antigen for preventing infection with *Streptococcus pneumoniae* — was intranasally administered with mCT, antigen-specific mucosal IgA and systemic IgG responses were elicited[90]. Mice intranasally immunized with PspA and mCT were also protected against a lethal challenge with *S. pneumoniae*[90]. Interestingly, when the tetanus-toxoid vaccine (which is currently administered by injection) was intranasally administered with one of these two mCTs, it generated protective immunity against challenge with the toxin[91]. An independent study has also shown that mCT E112K is the safest and most effective of the currently available toxin-based mutant adjuvants[92]. Taken together, these findings support the idea that mCT is a strong candidate for an effective mucosal adjuvant to generate protective immunity by the nasal route of administration. Indeed, these findings indicate that the current preference for injection-type vaccines should be reconsidered, and in future, greater use should be made of spray-type vaccines that include mCT and other safe toxin-based adjuvants (BOX 1).

To further enhance the efficacy of the mCT mucosal adjuvant, a second-generation, chimeric-type adjuvant was constructed from the A subunit of mCT (mCTA) and the B subunit of nLT (nLTB); therefore, the adjuvant has the immunobiological properties of both cholera toxin and heat-labile enterotoxin[93]. Nasal immunization with influenza-virus haemagglutinin plus the newly created chimeric mucosal adjuvant mCTA–nLTB resulted in significant haemagglutinin-specific serum IgG and IgA responses[93] (TABLE 2). In addition, mice that were intranasally immunized with haemagglutinin and mCTA–nLTB showed high levels of haemagglutinin-specific IgA in nasal and lung washes and were protected from viral challenge[93]. These findings show that nasal vaccines containing mCT or mCTA–nLTB are effective for the induction of protective immunity. The goal of mucosal-vaccine development cannot be realized without the creation of such novel and safe mucosal adjuvants.

Box 1 | **Advantages and disadvantages of nasal vaccination**

Advantages

- **Is the most effective route to elicit optimal protective immunity in both mucosal and systemic immune compartments.**
- **Can effectively induce antigen-specific immunity in the reproductive tract, as well as in the upper respiratory tract.**
- **Can generate cross-protective immunity in the gut through the common mucosal immune system.**
- **Can avoid degradation of vaccine antigen caused by digestive enzymes, so requires a smaller dose of antigen than oral immunization.**
- **Does not require injection, so is less painful.**
- **Does not require trained medical personnel for delivery.**

Disadvantages

- **Possible deposition of antigen in the central nervous system through the olfactory bulbs and olfactory nerves; this requires further investigation.**
- **Requires adjuvant safety to be clinically determined; clinical studies indicate that Bell's palsy is caused by influenza nasal vaccine that contains the native form of *Escherichia coli* heat-labile enterotoxin as a mucosal adjuvant.**

BELL'S PALSY
Facial paralysis that is thought to be triggered by viral infection. The facial nerve is oedematous in patients suffering from this disease.

Recent progress in clinical application of nasal vaccination. Between the late 1950s and the early 1960s, the efficacy of immunization with an intranasally administered vaccine against infection with influenza virus was shown in a large clinical trial in Osaka, Japan, in which a nasal-spray vaccine containing live attenuated influenza virus was administered to more than 10,000 volunteers[94,95]. In recent years, two types of intranasally administered influenza vaccine, an inactivated form and a live attenuated form, were introduced in Switzerland and the United States respectively. Indeed, as early as 1997, an inactivated form of nasal vaccine containing a small amount of nLT as a mucosal adjuvant was introduced in Switzerland. However, this influenza vaccine was withdrawn from the market because of the development of BELL'S PALSY by some recipients after nasal vaccination. A causal relationship between the intranasally administered inactivated-influenza vaccine used in Switzerland and the incidence of Bell's palsy was formally established in a recent case–control study[96]. At this stage, the causes and pathogenesis of Bell's palsy remain unclear; however, because nLT has been shown to have pro-inflammatory properties and possible neurological toxicity[97], the co-formulated nLT that is present in the inactivated-influenza vaccine is suspected to be the causative agent[98]. These findings highlight that the development of a safe mucosal adjuvant is crucial if progress is to be made towards a safe and effective mucosal vaccine.

In the past year, on the basis of promising clinical trials showing the induction of protective immunity, an intranasally administered cold-adapted influenza vaccine, known as 'FluMist', has been made available to healthy Americans of ages 5 to 49 (REFS 99,100). It should be noted that the concept of cold-adapted influenza virus was reported in 1967 (REF. 101), so more than 35 years were required for this discovery to be translated into an intransal vaccine against infection with influenza virus. More recently, the concept of nasal immunization was adopted for the development of a vaccine against severe acute respiratory syndrome (SARS). An experimental nasally administered vaccine against SARS that consists of a recombinant attenuated parainfluenza virus expressing the envelope spike protein of the SARS coronavirus was able to induce protective immunity in African green monkeys, including SARS-coronavirus-specific neutralizing antibodies[102]. Although further experiments are essential before this finding can be applied to developing a vaccine against SARS for use in humans, the study emphasizes the usefulness of mucosal immunization for the immunoprophylaxis of infectious diseases.

Concluding remarks

The mucosal immune system is now recognized to be an important first line of defence against invading pathogens. NALT and Peyer's patches are important inductive sites for the initiation of antigen-specific mucosal IgA and serum IgG responses, as well as CTL immune responses, at both mucosal and systemic sites; in this way, both NALT and Peyer's patches function to maximize the two-tiered immunological barrier of the host. The respiratory mucosal immune system has several immunological characteristics that are distinct from those of the Peyer's-patch-centred intestinal mucosal immune system. Although the tissue-genesis programme for other secondary lymphoid tissues, including Peyer's patches, begins during embryonic life, the NALT-organogenesis programme is initiated only after birth. Lymphoid organogenesis of Peyer's patches requires cytokine-mediated programmed inflammation (through the LT-βR) and signalling through the IL-7R, whereas the initiation of NALT development seems to be independent of the IL-7R, LT-$\alpha_1\beta_2$ and the LT-βR. Although the inducer cells for both NALT and Peyer's patches have a common phenotype, that is, $CD3^-CD4^+CD45^+$, NALT-inducer cells seem to be regulated by ID2 alone, whereas Peyer's-patch-inducer cells depend on both ID2 and ROR-γ. Because the organogenesis programme of NALT is different from that of other secondary lymphoid tissues, such as Peyer's patches, efforts should now be aimed at elucidating the distinct molecular characteristics of the NALT-genesis programme and the functional consequences of this. Clearly, it is important to have a thorough understanding of the unique molecular and cellular properties of the NALT-centred mucosal immune system for the development of a successful nasal vaccine.

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Competing interests statement

The authors declare no competing financial interests.

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