T-CELL TRAFFICKING IN ASTHMA: LIPID MEDIATORS GREASE THE WAY

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Abstract | Recruitment of T cells to the airways is crucial in the pathogenesis of asthma, and it is thought to be mediated mainly by peptide chemokines. By contrast, lipid mediators such as leukotrienes and prostaglandins have classically been thought to contribute to asthma pathogenesis by other mechanisms. However, as we discuss here, the recent molecular identification of leukotriene and prostaglandin receptors, as well as the generation of mice that are genetically deficient in them, has revealed that two of these lipids — leukotriene B_4 and prostaglandin D_2 — also direct T-cell migration and seem to cooperate with chemokines in a non-redundant, sequential manner to recruit T cells to the airways in asthma.

ALLERGIC PULMONARY INFLAMMATION
Lung inflammation induced by immediate and delayed hypersensitivity reactions to air-borne antigens. Allergic pulmonary inflammation is characterized pathologically by T helper 2 cell and eosinophil infiltration, and mucus hypersecretion, and physiologically by hyper-responsiveness to bronchoconstricting stimuli.

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Asthma is a disease of chronic airway inflammation that is characterized by eosinophilic infiltration, mucus hypersecretion and airway hyper-responsiveness (AHR). This inflammation produces intermittent airway obstruction, which is characterized by wheezing and shortness of breath[1]. Evidence from patients with asthma and from mouse models of this disease indicates that $CD4^+$ T helper 2 (T_H2) cells have a crucial role in mediating the inflammation that underlies asthma[2]. However, the molecular mechanisms that control the trafficking of T_H2 cells to the lungs in asthma are unknown.

Eicosanoids — named after the Greek 'eicosa' meaning twenty, which refers to the number of carbon atoms they contain — are biologically active lipids that are rapidly generated at sites of inflammation[3]. Leukotrienes and prostaglandins are two classes of eicosanoid that are thought to be involved in asthma pathogenesis: compared with individuals who do not suffer from asthma, the levels of these eicosanoids have been noted to be increased in the airways of patients with asthma. These molecules have classically been thought to be important mediators of the early phase of the asthmatic response to inhaled allergens[2]. However, as we discuss here, the recent discovery that the leukotriene B_4 (LTB_4) receptor BLT1 and the prostaglandin D_2 (PGD_2) receptor DP2 (also known as chemoattractant-receptor homologous molecule expressed by T_H2 cells, CRTH2) are expressed by specific classes of T cell, and recent experiments using mice that are deficient in BLT1 or the other PGD_2 receptor, DP1, have revealed important unexpected roles for these receptors and their eicosanoid ligands in the T-cell trafficking that occurs in ALLERGIC PULMONARY INFLAMMATION. On the basis of these experiments, we propose a model of T_H2-cell trafficking in asthma, in which the lipid mediators LTB_4 and PGD_2 direct the earliest phases of T-cell recruitment to the airways immediately after exposure to allergens, whereas peptide chemokines direct the subsequent phases of T-cell recruitment that amplify and/or maintain airway inflammation in asthma.

Generation of LTB_4 and PGD_2

Lipid mediators, such as LTB_4 and PGD_2, are not stored and released but, instead, are synthesized *de novo* after the activation of cells by mechanical trauma, bacterial peptides, allergens, or inflammatory mediators such as cytokines and growth factors[3]. Eicosanoids are derived from arachidonic acid, a polyunsaturated fatty acid (eicosatetra-5,8,11,14-enoic acid; 20:4ω6) that is kept ESTERIFIED to membrane phospholipids until it is mobilized by phospholipases, including cytosolic phospholipase A_2 (PLA_2) and secretory PLA_2 (FIG. 1). The enzymes that are involved in the generation of LTB_4 and PGD_2 from arachidonic acid are outlined in TABLE 1.

Biosynthesis of LTB_4. Leukotrienes are generated by the metabolism of arachidonic acid through the 5-lipoxygenase (5-LO) pathway[3]. Following cellular activation, 5-LO is translocated to the nuclear membrane[4], where it receives arachidonic acid donated by the integral nuclear-membrane protein known as 5-LO-activating protein (FLAP) and sequentially generates 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then the unstable intermediate LTA_4 (FIG. 1). LTA_4 is metabolized to either LTB_4, by LTA_4 hydrolase (LTA_4H), or to cysteinyl leukotrienes — including LTC_4, LTD_4 and LTE_4 — by LTC_4 synthase (LTC_4S). Although LTA_4H and LTC_4S are widely expressed by many tissues, 5-LO expression is generally restricted to myeloid cells[5,6] — particularly neutrophils, eosinophils, monocytes/macrophages and mast cells — which confers the ability to independently generate leukotrienes specifically to these cells. Other cell types have been shown to be able to generate leukotrienes but only from LTA_4 that is delivered by myeloid leukocytes — a process known as transcellular biosynthesis[7].

Biosynthesis of PGD_2. Prostaglandins are generated by the metabolism of arachidonic acid through the cyclooxygenase (COX) pathway[3]. Following cellular activation, arachidonic acid that is released by PLA_2 is presented to COX1 (also known as prostaglandin H synthase 1, PGHS1) and COX2 (PGHS2), which are present in the endoplasmic reticulum and nuclear membrane (FIG. 1). The COX enzymes generate the prostaglandin intermediate PGH_2, which is then metabolized to PGD_2, PGE_2, PGF_2, PGI_2 or thromboxane A_2 (TXA_2) in a cell-specific manner[3]. PGH_2 is converted to PGD_2 by two types of PGD synthase (PGDS)[8]: LIPOCALIN-type PGDS (L-PGDS), which is expressed by cells of the central nervous system, epididymis and

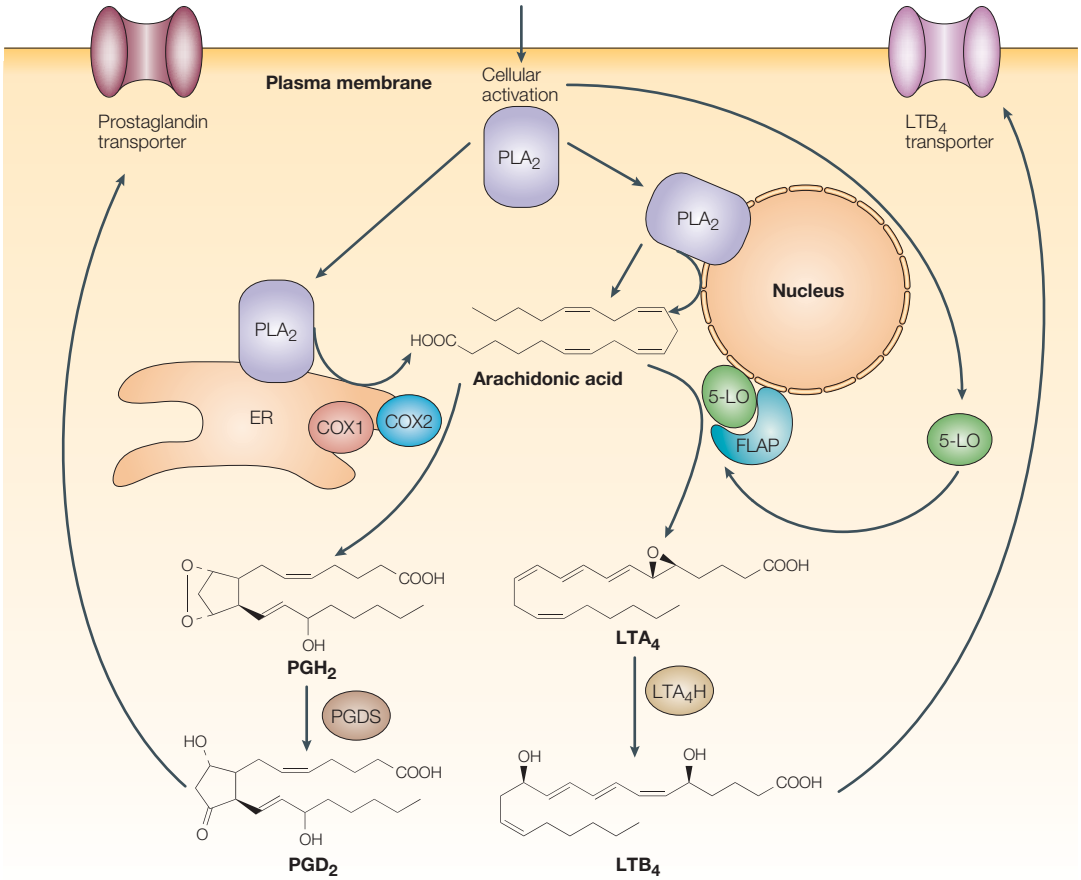

Figure 1 | **Generation of LTB_4 and PGD_2.** Lipid mediators, such as LTB_4 and PGD_2, are synthesized *de novo* when cells such as mast cells and macrophages are activated by various inflammatory stimuli, including mechanical trauma, bacterial peptides, allergens, cytokines and growth factors. These stimuli trigger the translocation of cytosolic phospholipase A_2 (PLA_2) to the endoplasmic reticulum (ER) and the nuclear membrane, where it releases arachidonic acid from membrane phospholipids. Leukotrienes, including leukotriene B_4 (LTB_4), are generated from released arachidonic acid through the 5-lipoxygenase (5-LO) pathway. Following cellular activation, 5-LO translocates to the nuclear membrane, where it receives arachidonic acid from 5-LO-activating protein (FLAP) (a resident nuclear-membrane integral protein that seems to function as an arachidonic-acid-transfer protein) and generates the common leukotriene intermediate LTA_4. LTA_4 is then metabolized to LTB_4 by LTA_4 hydrolase (LTA_4H), and efflux of the produced LTB_4 is facilitated by an as-yet-unidentified transporter. Prostaglandins, including prostaglandin D_2 (PGD_2), are generated from released arachidonic acid through the cyclooxygenase (COX) pathway. Following cellular activation, arachidonic acid is metabolized to the common prostaglandin intermediate PGH_2 by COX1 and COX2, which are present in the ER and nuclear membrane. PGH_2 is then converted to PGD_2 by PGD synthase (PGDS), and efflux of the generated PGD_2 is facilitated by a prostaglandin transporter.

ESTERIFIED
Combination of one organic compound with another through the formation of an ester: that is, the condensation of a hydroxyl group of one compound with a carboxyl group of another.

LIPOCALIN
Member of a large superfamily of small, mostly extracellular proteins of diverse function. Lipocalins have two common features: an eight-stranded antiparallel β-sheet closed back on itself to form a continuously hydrogen-bonded β-barrel; and the ability to bind small, mainly hydrophobic molecules.

testis; and haematopoietic PGDS (H-PGDS), which is expressed by immune and inflammatory cells, such as mast cells[9], macrophages[10], dendritic cells[10] and T_H2 cells[11]. PGD_2 is the main COX product that is generated by mast cells activated by crosslinking of the high-affinity receptor for IgE (FcεRI) (REFS 12,13).

Receptors for LTB_4 and PGD_2

Two receptors have been molecularly identified for each of LTB_4 and PGD_2: BLT1 and BLT2, and DP1 and DP2, respectively. All four receptors are members of the G-protein-coupled seven-transmembrane-domain receptor superfamily, but they differ in their ligand affinities and patterns of expression (TABLE 2).Two of these receptors, BLT1 and DP2, are highly expressed by specific T-cell subsets. As we discuss here, BLT1 expression by T cells has been shown to mediate LTB_4-directed T-cell trafficking in mouse models of asthma, whereas DP2, although expressed by T cells, has not yet been shown to mediate PGD_2-directed T-cell trafficking in asthma. Interestingly, DP1, although not expressed by T cells, has been shown to mediate PGD_2-directed T-cell trafficking through the induction of chemokine expression.

T-cell expression of BLT1. Although LTB_4 was classically described as a chemoattractant for myeloid leukocytes[14,15], even before the identification of LTB_4 receptors, it was shown that LTB_4 could bind specifically to a small proportion of human peripheral-blood T cells[16] and to the T-lymphoblastoma cell line Tsup-1 (REF. 17). Consistent with these findings, LTB_4 was shown to induce chemotaxis of human peripheral-blood lymphocytes[18] and Tsup-1 cells[17], and topical application of LTB_4 on the skin of healthy volunteers was shown to cause infiltration of T cells *in vivo*[19]. Definitive description of LTB_4-receptor expression by T cells followed the molecular identification of BLT1 (REFS 20,21), which was noted to be highly expressed by T-cell lymphomas[21]. High levels of BLT1 were subsequently noted to be expressed by mouse $CD4^+$ (REF. 22) and $CD8^+$ (REFS 23,24) T cells that have been differentiated *in vitro* to effector phenotypes. $CD4^+$ T cells that were activated *in vitro* under non-polarizing (T_H0), T_H1-polarizing or T_H2-polarizing conditions all had increased levels of mRNA encoding BLT1 compared with naive cells, which expressed little BLT1 (REF. 22) (FIG. 2). By contrast, the expression of BLT2 by naive T cells or by T_H0, T_H1 or T_H2 effector cells was not detected. BLT1 expression has also been shown to be induced in $CD4^+$ T cells that leave the lymph node and enter the tissue after activation by antigen *in vivo* in intact mice[22].

Marked induction of BLT1 expression has also been observed in $CD8^+$ T cells that are activated *in vitro*. However, in contrast to $CD4^+$ T cells, in which BLT1 expression is upregulated by all subsets of antigen-experienced cells, different subsets of antigen-experienced $CD8^+$ T cells show differential induction of BLT1 expression. Mouse splenic $CD8^+$ T cells that are activated *in vitro* in the presence of interleukin-15 (IL-15) have been shown to resemble CENTRAL MEMORY T (T_{CM}) CELLS, whereas $CD8^+$ T cells that are activated in the presence of IL-2 resemble EFFECTOR MEMORY T CELLS and effector T cells (collectively denoted as T_{EFF}, in this review)[25,26]. Naive $CD8^+$ T cells and $CD8^+$ T_{CM} cells expressed little mRNA encoding BLT1, whereas BLT1

Table 1 | **Enzymes involved in LTB_4 and PGD_2 synthesis**

Enzyme	Substrate	Product	Expression
Synthesis of both LTB_4 and PGD_2			
Cytosolic and secretory PLA_2	Esterified arachidonic acid	Arachidonic acid	Widely expressed by most cells and tissues
Synthesis of LTB_4			
5-lipoxygenase	Arachidonic acid	LTA_4	Mast cells, neutrophils, eosinophils, basophils, monocytes/macrophages, B cells, dendritic cells and pulmonary endothelial cells in inflammatory conditions
LTA_4 hydrolase	LTA_4	LTB_4	Cells expressing 5-lipoxygenase, erythrocytes, platelets, T cells, keratinocytes, airway epithelial cells, intestinal epithelial cells and fibroblasts; heart, kidney, adrenal cortex and seminal vesicles
Synthesis of PGD_2			
Cyclooxygenase-1	Arachidonic acid	PGH_2	Constitutively expressed by most cells and tissues
Cyclooxygenase-2	Arachidonic acid	PGH_2	Widely induced by cytokines and mitogens
Haematopoietic PGD synthase	PGH_2	PGD_2	Mast cells, macrophages, dendritic cells and T helper 2 cells
Lipocalin-type PGD synthase	PGH_2	PGD_2	Central nervous system, epididymis and testis

LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; PGD_2, prostaglandin D_2; PGH_2, prostaglandin H_2; PLA_2, phospholipase A_2.

CENTRAL MEMORY T (T_{CM}) CELLS
Antigen-experienced $CD8^+$ T cells that lack immediate effector function but are able to mediate rapid recall responses. They also rapidly develop the phenotype and function of effector memory cells after restimulation with antigen. Central memory T cells retain the migratory properties of naive cells and therefore circulate through the secondary lymphoid organs.

EFFECTOR MEMORY T CELLS
Antigen-experienced $CD8^+$ T cells that have immediate effector capabilities, such as cytotoxicity, and can efficiently migrate to peripheral sites of inflammation.

Table 2 | **Receptors for LTB_4 and PGD_2**

Receptor	Ligand	Expression
BLT1	LTB_4 (K_d ~0.5 nM)	Effector $CD4^+$ and $CD8^+$ T cells, neutrophils, eosinophils, basophils and monocytes/macrophages; lung, spleen and lymph node
BLT2	LTB_4 (K_d ~23 nM) and hydroxyeicosanoids	Widely expressed by most human tissues
DP1	PGD_2 (K_d ~1.5 nM)	Airway epithelial cells and basophils; spinal cord, brain, retina, digestive system
DP2	PGD_2 (K_d ~2.5 nM)	T helper 2 cells, eosinophils, basophils and monocytes; digestive system, heart, thymus, spinal cord and brain

K_d, rate of dissociation; LTB_4, leukotriene B_4; PGD_2, prostaglandin D_2.

expression was substantially upregulated by $CD8^+$ T_{EFF} cells[23] (FIG. 2); gene-array analysis also revealed that the level of BLT1 expression was one of the most marked differences between $CD8^+$ T_{EFF} and T_{CM} cells[24]. Similar to $CD4^+$ T cells, no $CD8^+$ T-cell subset was found to express BLT2 (REF. 23).

T-cell expression of DP2. Before the discovery was made that PGD_2 is the natural ligand of DP2, this receptor was cloned as an orphan chemoattractant G-protein-coupled receptor, and the mRNA encoding DP2 was shown to be expressed by human T_H2 cells but not T_H1 cells[27]. The specific expression of DP2 by T_H2 cells, but not T_H1 cells, has now been confirmed at the protein level[27,28] (FIG. 2). In some *in vitro*-differentiated T_H2-cell clones that express DP2, addition of IL-4 to the culture medium increased receptor expression, whereas treatment with IL-12 reduced DP2 expression, indicating that DP2 expression is regulated by both T_H1 and T_H2 cytokines[27]. In fresh peripheral-blood mononuclear cells (PBMCs) from healthy adults, a small population of $CD4^+$ T cells (0.4–6.5% of PBMCs) were shown to express DP2 in all donors, and most of these cells were $CD25^+CD45RA^-CD45RO^+$, which indicates an activated antigen-experienced phenotype. Furthermore, more than 85% of circulating DP2-expressing $CD4^+$ T cells produced IL-4, IL-5 and/or IL-13, but little interferon-γ (IFN-γ), indicating that DP2 is selectively expressed by T_H2 cells *in vivo*, as well as by T_H2 cells that have been differentiated *in vitro*[27]. A comparison of DP2 expression and chemokine-receptor expression by T_H2 cells present in PBMCs from healthy donors[29] showed that DP2 expression identified T_H2 cells more reliably than expression of CC-chemokine receptor 3 (CCR3) or CCR4, two chemokine receptors that were previously noted to be preferentially expressed by T_H2 cells[30–32].

In addition to its expression by T_H2 cells from healthy volunteers, DP2 is expressed by T_H2 cells in pathological inflammatory conditions. In PBMCs that are isolated from individuals with sensitivity to pollen, depletion of $DP2^+$ cells markedly reduced proliferative responses to pollen allergens, indicating that most pollen-allergen-responsive $CD4^+$ T cells expressed DP2 (REF. 27). Similarly, among dust-mite-antigen-stimulated PBMCs from individuals that have high serum concentrations of IgE specific for mite allergens, most T_H2-cytokine-producing $CD4^+$ T cells expressed DP2 (REF. 33). For patients with atopic dermatitis, a skin disease that is associated with increased T_H2-cell responses[34], a higher proportion of the PBMC population consists of DP2-expressing $CD4^+$ T cells than for control individuals[29]. Furthermore, among patients with atopic dermatitis, the percentage of $CD4^+$ T cells that express DP2 correlated with the severity of disease[33]. Together, these results show that DP2 is expressed by antigen-specific T_H2 cells developing *in vivo* in allergic humans, and they provide support for the idea that this receptor might be an important contributor to the recruitment of T_H2 cells in allergic diseases.

DP2 is also expressed by a small percentage of $CD8^+$ T cells from some, but not all, healthy donors[27], particularly by $CD8^+$ T cells that produce the cytokines IL-4 and IL-13 (REF. 29) similar to DP2-expressing $CD4^+$ T cells. In contrast to DP2 expression, T cells were not noted to express DP1, but DP1 is expressed by basophils[28] and airway epithelial cells (TABLE 2). Expression of DP1 by airway epithelial cells has been shown to be crucial for PGD_2-directed T-cell trafficking in mouse models of asthma, through the induction of chemokines (discussed later).

Functional responses of T cells to LTB_4 and PGD_2

T-cell responses to LTB_4. LTB_4 mediates the recruitment of myeloid leukocytes to sites of inflammation, both by directing myeloid leukocyte migration[14,15] and by altering the nature of myeloid leukocyte–endothelial cell interactions from those that support LEUKOCYTE ROLLING to those that support LEUKOCYTE FIRM ADHESION[35,36]. After the finding that mouse $CD4^+$ effector T cells express high levels of BLT1, LTB_4 was found to potently induce both chemotaxis and firm adhesion of these cells (FIG. 3). Indeed, LTB_4 was shown to induce dose-dependent chemotaxis of *in vitro*-polarized mouse T_H1 and T_H2 cells[22]. The magnitude of the $CD4^+$ effector T-cell chemotactic responses to LTB_4 was comparable to that induced by CXC-chemokine ligand 12 (CXCL12; also known as SDF1), one of the most efficacious T-cell chemokines[37]. By contrast, LTB_4 did not induce chemotaxis of naive $CD4^+$ T cells, indicating that the low level of BLT1 expressed by these cells is not functional with respect to chemotaxis. Further studies using T_H1 and T_H2 cells derived from BLT1-deficient mice[38] showed that these cells did not migrate in response to LTB_4, thereby indicating that LTB_4-induced chemotaxis of $CD4^+$ effector T cells is mediated by BLT1 (FIG. 3).

Firm adhesion in blood vessels is required before leukocytes can migrate to tissues. In most settings, rolling leukocytes must encounter a chemotactic stimulus that triggers rapid activation of integrins, which mediate firm arrest (FIG. 3). LTB_4 was shown to induce firm arrest of rolling mouse T_H1 and T_H2 cells *in vitro* to an extent comparable to CXCL12 (REF. 22). This effect was absent in T_H1 and T_H2 cells that were derived from BLT1-deficient mice, indicating that LTB_4-induced firm

LEUKOCYTE ROLLING
The initial interactions that occur between circulating leukocytes and the endothelial cells of inflamed tissues are transient low-affinity adhesive interactions that are mediated by the selectin family of adhesion molecules, resulting in the rolling of leukocytes along the endothelial surface. The process of rolling slows leukocytes to velocities less than those of circulating erythrocytes.

LEUKOCYTE FIRM ADHESION
The interactions of rolling leukocytes with chemokines or lipid mediators, such as leukotriene B_4, at the endothelial surface lead to the activation of leukocyte integrins — another family of adhesion molecules. When activated, integrins mediate high-affinity adhesive interactions between leukocytes and endothelial cells, resulting in the arrest and firm adhesion of rolling leukocytes.

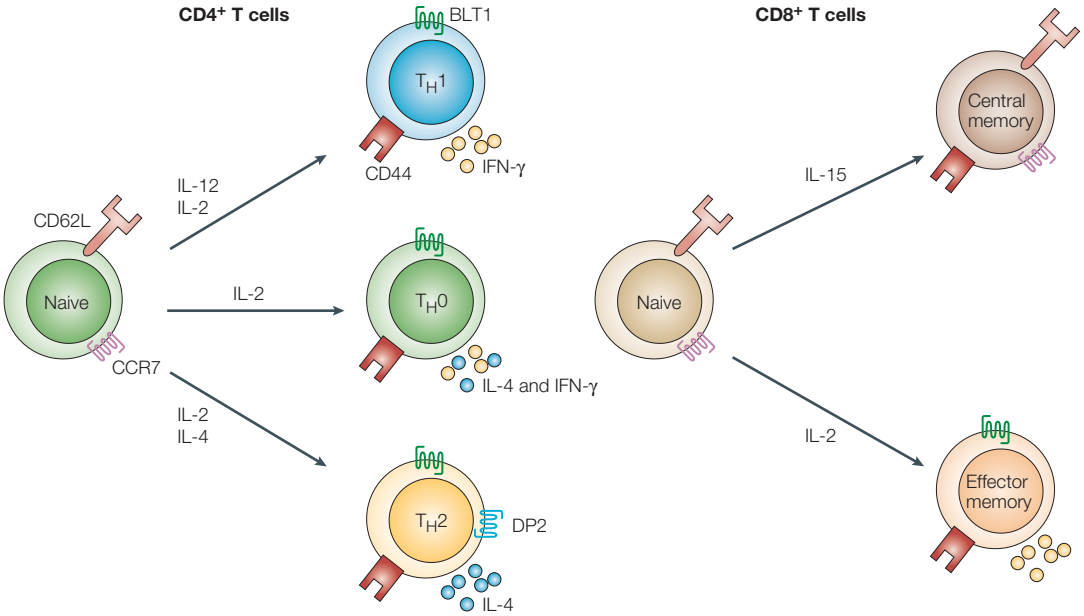

Figure 2 | **T-cell expression of the receptors for LTB_4 and PGD_2.** Both naive $CD4^+$ and $CD8^+$ T cells express CD62 ligand (CD62L) and CC-chemokine receptor 7 (CCR7), which direct their trafficking to secondary lymphoid tissues. They also both express low levels of the leukotriene B_4 (LTB_4) receptor BLT1, but they do not respond to LTB_4. After encounter with cognate antigen, activation of naive $CD4^+$ T cells induces their differentiation into $CD4^+$ effector T cells, a process marked by downregulation of CD62L and CCR7 expression and upregulation of the expression of activation markers such as CD44. Antigen-experienced $CD4^+$ effector T cells are functionally classified by their cytokine expression, with T helper 1 (T_H1) cells producing interferon-γ (IFN-γ), T_H2 cells producing interleukin-4 (IL-4), IL-5 and IL-13, and T_H0 cells producing both T_H1 and T_H2 cytokines. Regardless of whether they are differentiated under conditions that are T_H1-polarizing (in the presence of IL-12 and IL-2), T_H2-polarizing (IL-4 and IL-2) or non-polarizing (T_H0) (IL-2 only), all subsets of $CD4^+$ effector T cells express BLT1. Consequently, LTB_4 induces chemotaxis and firm arrest of all $CD4^+$ effector T-cell subsets. By contrast, among $CD4^+$ effector T-cell subsets, only T_H2 cells express DP2 (also known as chemoattractant-receptor homologous molecule expressed by T_H2 cells, CRTH2) and thereby respond to prostaglandin D_2 (PGD_2). Antigen-experienced $CD8^+$ T cells are phenotypically classified by their functional and migratory characteristics: $CD8^+$ central memory T (T_{CM}) cells home preferentially to lymphoid organs, consistent with their retention of CD62L and CCR7 expression; and $CD8^+$ effector memory and effector T cells (collectively denoted as T_{EFF}) are excluded from lymphoid tissues (except the spleen), consistent with their downregulation of CD62L and CCR7 expression. $CD8^+$ T_{EFF} cells, which migrate preferentially to inflamed tissues, express BLT1; consequently, LTB_4 induces chemotaxis and firm arrest of this subset of $CD8^+$ T cells. Interestingly, although $CD8^+$ T_{CM} cells express only low levels of BLT1, LTB_4 induces firm arrest of this $CD8^+$ T-cell subset, but it does not induce chemotaxis. DP2 has been found to be expressed by a small proportion of the peripheral-blood $CD8^+$ T cells of some human donors, although these cells have not been characterized as T_{CM} or T_{EFF} cells. Expression of the other known receptors for LTB_4 and PGD_2, BLT2 and DP1, has not been shown by any T-cell subset.

arrest of $CD4^+$ effector T cells is also mediated by BLT1. Recent studies indicate that chemoattractant-induced chemotaxis and arrest are distinct leukocyte functions that might be mediated by divergent signalling pathways[39], although further studies are required to determine whether this is the case for LTB_4 and T cells.

LTB_4 was also found to potently induce chemotaxis and firm adhesion of mouse $CD8^+$ T_{EFF} cells (FIG. 3). LTB_4 induced dose-dependent chemotaxis of *in vitro*-differentiated mouse $CD8^+$ T_{EFF} cells[23,24] as potently as the inflammatory chemokine CC-chemokine ligand 5 (CCL5; also known as RANTES), and studies with $CD8^+$ T_{EFF} cells derived from BLT1-deficient mice[38] showed that LTB_4-induced chemotaxis of $CD8^+$ T_{EFF} cells is mediated by BLT1 (REF. 23). By contrast, LTB_4 did not induce chemotaxis of $CD8^+$ T_{CM} or naive $CD8^+$ T cells, indicating that the low level of BLT1 expressed by these cells is not functional with respect to chemotaxis. Similar to T_H1 and T_H2 cells, LTB_4 induced firm arrest of $CD8^+$ T cells, as shown by INTRAVITAL MICROSCOPY STUDIES that compared the accumulation of adherent T cells in the presence and absence of LTB_4 (REF. 23). Interestingly, although LTB_4 did not induce chemotaxis of $CD8^+$ T_{CM} cells in venules super-perfused with LTB_4, it induced firm arrest of this T-cell subset with efficiency and kinetics similar to the firm arrest of $CD8^+$ T_{EFF} cells. By contrast, naive T cells did not arrest in venules exposed to LTB_4 super-perfusion. The induction of firm arrest by LTB_4 of both $CD8^+$ T_{EFF} and T_{CM} cells was entirely dependent on BLT1, because cells of either subset derived from BLT1-deficient mice failed to accumulate in response to LTB_4 super-perfusion. The differential responsiveness of different classes of $CD8^+$ T cell to LTB_4 is consistent with their varying migratory properties. $CD8^+$ T_{EFF} cells, which are the most responsive to LTB_4, migrate with the greatest efficiency to peripheral tissue sites of inflammation, where LTB_4 is produced. By contrast, $CD8^+$ T_{CM} cells migrate to

INTRAVITAL MICROSCOPY STUDIES
Examination of biological processes, such as leukocyte–endothelial cell interactions, in living tissue. In general, translucent tissues are used, such as the mesentery or cremaster muscle, which can be exteriorized and mounted for microscopic observation.

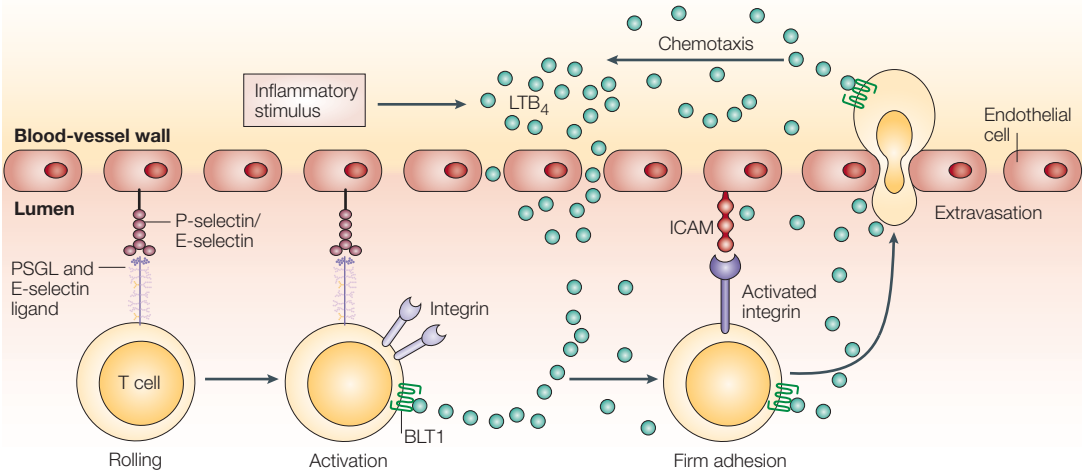

Figure 3 | **Mechanisms of effector T-cell recruitment mediated by LTB_4 and BLT1.** Leukocytes are recruited from the circulation to tissue sites of inflammation by a series of distinct processes that occur in the following sequence: rolling, activation, firm adhesion, extravasation and chemotaxis. The initial interactions occurring between leukocytes and the endothelium are transient low-affinity adhesions that are mediated by endothelial selectins and their ligands expressed by leukocytes; these result in the rolling of leukocytes along the endothelial surface. Leukocytes rolling on the endothelium encounter chemoattractants that engage specific leukocyte receptors, which induce intracellular signals that activate leukocyte integrins. Activated integrins then mediate high-affinity adhesive interactions between the leukocytes and the endothelium, by binding endothelial intercellular adhesion molecules (ICAMs), resulting in firm adhesion and arrest of rolling leukocytes. Subsequent to arrest, leukocytes extravasate through the endothelium to the tissue, where they migrate to sites of inflammation in response to chemoattractant concentration gradients. Leukotriene B_4 (LTB_4), which was traditionally described as a myeloid-cell chemoattractant, has now been shown to induce chemotaxis of $CD4^+$ effector T cells —both T helper 1 (T_H1) and T_H2 cells — and of $CD8^+$ effector memory T cells and $CD8^+$ effector T cells (collectively denoted as $CD8^+$ T_{EFF} cells). It also induces the firm adhesion of rolling $CD4^+$ effector T cells, $CD8^+$ T_{EFF} cells and $CD8^+$ central memory T cells, by activating the LTB_4 receptor BLT1. E-selectin, endothelial-cell selectin; P-selectin, platelet selectin; PSGL, P-selectin glycoprotein ligand 1.

peripheral inflammatory sites with substantially lower efficiency than $CD8^+$ T_{EFF} cells, and naive $CD8^+$ T cells are not recruited to peripheral tissues[26].

T-cell responses to PGD_2. Following the identification of PGD_2 as a specific ligand of DP2, PGD_2 was shown to induce both mobilization of calcium ions and chemotaxis of T_H2 cells, but not T_H1 cells, that were differentiated *in vitro* from PBMCs of healthy adults[28]. Both PGD_2-induced calcium mobilization and T_H2-cell chemotaxis were almost completely inhibited by a neutralizing monoclonal antibody specific for DP2, whereas a DP1-specific agonist failed to induce calcium mobilization or chemotaxis, indicating that these functional effects of PGD_2 on T_H2 cells are mediated through DP2, rather than DP1 (REF. 28). PGD_2 also induced chemotaxis of freshly isolated $DP2^+CD4^+$ T cells but not $DP2^-CD4^+$ T cells[33]. The ability of PGD_2 to induce firm arrest of rolling T_H2 cells has not been investigated, although PGD_2 has been noted to induce upregulation of expression of the β_2-integrin CD11b by $DP2^+$ human eosinophils[40].

Roles of LTB_4 and PGD_2 in asthma

Increased levels of LTB_4 and PGD_2 are present in the airways of patients with asthma compared with those of individuals who do not suffer from asthma. Furthermore, in experimental models of asthma, rodents inhaling a specific antigen that they have been immunized against generate both LTB_4 (REF. 41) and PGD_2 (REFS 42,43) in the airways. Although LTB_4 and PGD_2 have classically been considered to have roles in asthma that are not associated with the recruitment of T cells, the generation of BLT1- and DP1-deficient mice has now allowed investigators to uncover important new roles for these receptors and their eicosanoid ligands in the T-cell trafficking that occurs in allergic pulmonary inflammation.

Classical roles of LTB_4 in asthma. Increased expression levels of 5-LO and LTA_4H have been noted in the airways[44] and circulating neutrophils[45] of patients with asthma. In addition, increased levels of LTB_4 have been found in the blood[46,47], BRONCHOALVEOLAR LAVAGE (BAL) fluid[48,49] and exhaled breath condensates[50,51] of patients with asthma. Whereas the cysteinyl leukotrienes are potent mediators of BRONCHOCONSTRICTION[52], the classical pathogenic activities attributed to LTB_4 are the recruitment[15,53], activation[54,55] and prolongation of survival[56] of myeloid leukocytes, including neutrophils and eosinophils. A pathogenic role for neutrophils in asthma has been indicated by the large number of neutrophils in the airways of patients with asthma who are suffering clinical exacerbations[57,58] or status asthmaticus[58], and in those individuals who have undergone a sudden asthma-related death[59]. A pathogenic role for eosinophils in asthma has been supported by a correlation between eosinophil numbers in the airways and disease severity[60], although AHR was not affected by reductions in the number of airway eosinophils following treatment

BRONCHOALVEOLAR LAVAGE
Delivery of saline to the airways and airspaces of the lungs, followed by retrieval of the fluid. This procedure is carried out to obtain samples of the cells, proteins or other materials that line the aerated regions of the lungs.

BRONCHOCONSTRICTION
Contraction of smooth muscle that surrounds the airways, resulting in airway narrowing and air-flow obstruction, which produces symptoms of wheezing, shortness of breath and chest tightness. In asthma, bronchoconstriction is thought to be mediated by inflammatory mediators, including prostaglandins and leukotrienes, and it can usually be reversed using β-adrenergic agonist or anticholinergic medications.

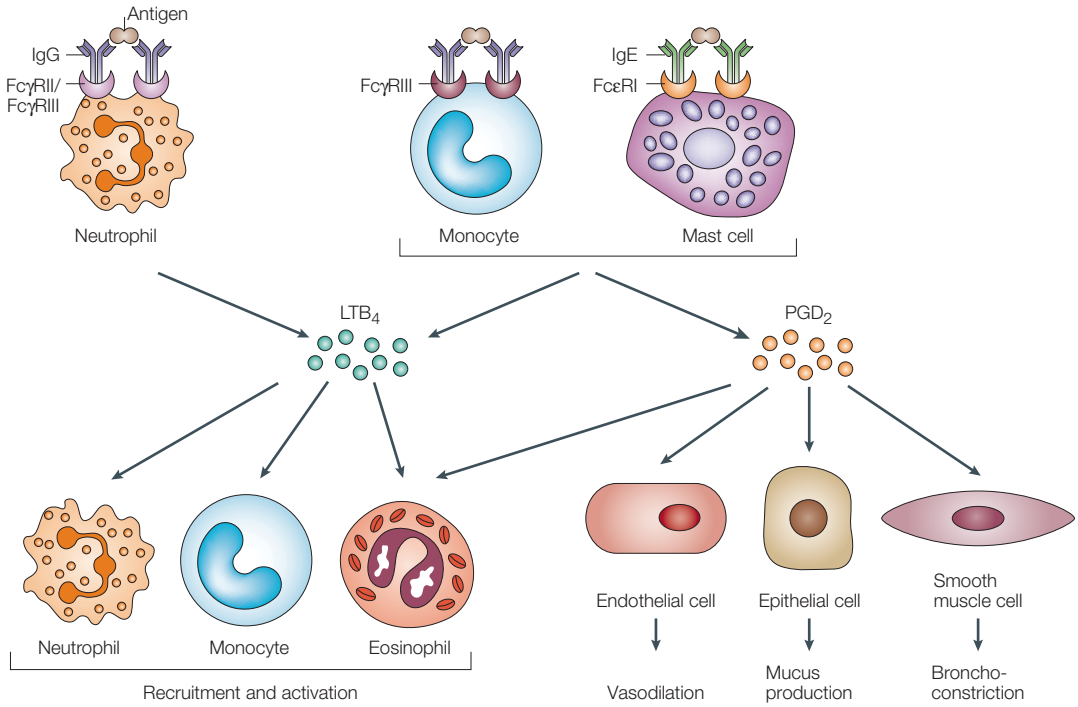

Figure 4 | **Classically described activities of LTB_4 and PGD_2 that are relevant to asthma.** When activated by antigen-induced Fc receptor crosslinking, mast cells, monocytes/macrophages and neutrophils produce leukotriene B_4 (LTB_4), and mast cells and monocytes/macrophages produce prostaglandin D_2 (PGD_2). Classically described activities of LTB_4 that might contribute to asthma pathogenesis include the recruitment and activation of myeloid leukocytes, such as neutrophils, monocytes and eosinophils. Classically described activities of PGD_2 that might participate in asthma include vasodilatation and increased capillary permeability, leading to oedema formation, increased mucus production, bronchoconstriction and eosinophil recruitment. FcγRII, low-affinity Fc receptor for IgG; FcγRIII, low-affinity Fc receptor for IgG; FcεRI, high-affinity receptor for IgE.

of patients with asthma using an IL-5-specific monoclonal antibody (known as mepolizumab)[61]. So, LTB_4 might contribute to pathogenesis in asthmatic airways through the recruitment and activation of neutrophils and eosinophils (FIG. 4).

Classical roles of PGD_2 in asthma. Increased levels of PGD_2 have also been found in the BAL fluid of patients with asthma[62,63], and PGD_2 levels in BAL fluid from atopic asthmatic patients have been shown to increase considerably following airway allergen challenge[64,65]. The classically described activities of PGD_2 that might participate in asthma pathogenesis include vasodilation[66] and increased capillary permeability[67], increased mucus production[68] and bronchoconstriction (FIG. 4). Inhalation of PGD_2 induces bronchoconstriction in both patients with asthma and in normal individuals, with asthma patients being more responsive to PGD_2-induced bronchoconstriction than control individuals[69,70]. Additionally, administration of PGD_2 to the canine airway has been shown to induce the accumulation of eosinophils[71], indicating that PGD_2 might also contribute to asthma pathogenesis in asthmatic airways through eosinophil recruitment (FIG. 4).

LTB_4-directed T-cell trafficking in asthma. A crucial role for LTB_4 in T-cell trafficking early in allergic pulmonary inflammation was recently shown following the generation of mice that are genetically deficient in BLT1 (REF. 22). In an active immunization mouse model of asthma, progressively increasing numbers of T cells were recruited to the airways and recovered in the BAL fluid of wild-type mice that were immunized with chicken ovalbumin (OVA) and subsequently administered sequential OVA aerosol challenges. After one or two challenges with OVA, the total number of T cells, as well as the number of both $CD4^+$ and $CD8^+$ T cells, was markedly decreased in the BAL fluid of BLT1-deficient mice compared with the BAL fluid of wild-type mice. However, following three challenges with OVA, similar numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells were present in the BAL fluid of BLT1-deficient and wild-type mice. Increased numbers of T cells were also recruited to the lungs of immunized mice after sequential aerosol challenges with OVA, but in contrast to the marked differences in BAL lymphocytes, similar numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells were present in the lung parenchyma of BLT1-deficient and wild-type mice after one, two or three challenges. These data indicate that BLT1 activation is required specifically for effector T-cell egress from the lungs to the airways, at early time points after antigen challenge[22] and that in the airways at these times, LTB_4 is the main chemoattractant directing effector T-cell recruitment.

In addition to cellular immune responses, immunization induces antigen-specific humoral responses. After aerosol challenge with OVA, OVA-specific antibodies that have been generated in immunized mice induce

the activation of airway mast cells and alveolar macrophages by Fc receptor crosslinking[72,73]. Because activated mast cells[74–76] and alveolar macrophages[77,78] generate LTB_4 in response to Fc receptor crosslinking, they are probably the source of LTB_4 in the airways at early time points following antigen challenge. Consequently, LTB_4 would be expected to be rapidly produced in the airways of immunized mice after aeroallergen challenge. The experiments described here using the active asthma model were carried out using wild-type and BLT1-deficient mice on the S129 genetic background. Interestingly, we have not seen a similar early defect in airway T-cell recruitment in analogous experiments carried out using BLT1-deficient mice on the C57BL/6 genetic background (B. D. Medoff, A.M.T. and A.D.L., unpublished observations). Whereas S129 mice have been noted to have measurable levels of OVA-specific IgE after immunization with OVA but before aerosol challenge with OVA, OVA-specific IgE levels have been reported to be undetectable in the serum of C57BL/6 mice at this time point[79]. Consequently, initial aerosol challenges of immunized C57BL/6 mice would not be expected to generate considerable levels of LTB_4 in the airways, and T-cell trafficking in wild-type and BLT1-deficient mice on this genetic background would be expected to be similar, as we have observed.

The finding that in BLT1-deficient mice, T-cell recruitment is reduced to the airways but not to the lung parenchyma is consistent with the hypothesis that activated airway mast cells and/or alveolar macrophages are the source of the LTB_4 that directs effector T-cell trafficking early in the active immunization model. Mast cells are present at the surface of and within the airways[80], and alveolar macrophages are, by definition, present in the airspaces. In mice that have generated OVA-specific antibody in reponse to previous immunization, exposure of the airways and alveoli to OVA delivered by aerosol challenge activates mast cells and macrophages that reside there, leading to the production of LTB_4 specifically in these locations. LTB_4 generated at airway–airspace surfaces would be predicted to direct effector T-cell trafficking to the airways and airspaces rather than to the lung parenchyma, consistent with the observations made using this model. The finding that BLT1 expression was specifically required for effector T-cell trafficking early in the active immunization model is also consistent with the rapidity with which airway mast cells and alveolar macrophages can generate LTB_4. In contrast to the peptide chemokines, the expression of which requires gene transcription and translation, LTB_4 is produced by sequential enzymatically catalysed reactions (as discussed earlier). So, whereas the expression of chemokines requires several hours, LTB_4 can be generated within minutes of cellular activation[81].

The hypothesis that LTB_4 generated by activated airway mast cells and/or alveolar macrophages directs early effector T-cell trafficking is supported by two other experimental observations: first, mast-cell activation by itself is sufficient to induce the recruitment of T_H2 cells to the airways; and second, stimulated mast cells induce the migration of effector T cells through production of LTB_4. In experiments that were designed to separate the effects that antigen-specific mast-cell activation and antigen-specific T_H2-cell activation have on T-cell recruitment, *in vitro*-differentiated T_H2 cells and IgE that recognized different antigens (OVA and the hapten dinitrophenol, DNP, respectively) were co-transferred to naive mice[82]. Following transfer, a single intranasal challenge with DNP-haptenated bovine serum albumin — which activated the transferred mast cells, but not the transferred T_H2 cells, through antigen–IgE immune-complex-mediated Fc receptor crosslinking — induced a marked influx of the T_H2 cells into the airways, indicating that mast-cell activation by itself is capable of mediating T_H2-cell recruitment. By contrast, a single challenge with OVA induced only a small influx of the transferred T_H2 cells into the airways.

Although activated mast cells secrete diverse effector molecules, LTB_4 has been shown to be the mast-cell mediator that directly induces the migration of effector T cells *in vitro*[24]. In transwell assays, mouse bone-marrow-derived mast cells (BMMCs) activated by Fc receptor crosslinking induced the migration of *in vitro*-differentiated $CD8^+$ T_{EFF} cells, which express high levels of BLT1, but not $CD8^+$ T_{CM} cells, which do not highly express BLT1. $CD8^+$ T_{EFF}-cell migration induced by activated BMMCs was blocked both by a specific inhibitor of FLAP, which prevents leukotriene production, and by a specific LTB_4 receptor antagonist, indicating that mast-cell-induced migration of $CD8^+$ T_{EFF} cells is mediated by LTB_4 and BLT1.

Consistent with the observations that airway T-cell recruitment is BLT1-dependent at early time points in the active immunization model and that mast-cell activation alone is sufficient for T_H2-cell recruitment early after allergen challenge, inflammatory-cell recruitment has been noted to be mast-cell dependent in asthma models that specifically use a small number of airway antigen challenges. After a single aeroallergen challenge, immunized cKIT-deficient (W/Wv) mice, which have congenitally low numbers of tissue mast cells, showed reduced eosinophilic inflammation[83]. By contrast, after three airway antigen challenges, immunized mast-cell-deficient mice showed inflammatory responses comparable to wild-type mice[84], analogous to the results obtained using BLT1-deficient mice[22]. These observations support the conclusion that early after the initiation of an allergic immune response, mast-cell mediators, such as LTB_4, direct T-cell trafficking, whereas later in the response, other chemoattractants that are generated by cells other than mast cells are sufficient to fully mediate effector T-cell trafficking.

PGD_2-directed T-cell trafficking in asthma. As for LTB_4, a crucial role for PGD_2 in the trafficking of T cells in allergic pulmonary inflammation was shown following the generation of mice that are genetically deficient in one of the PGD_2 receptors, DP1 (REF. 85). In an active immunization model consisting of systemic sensitization with OVA followed by aerosol OVA challenge, lymphocyte recruitment to the airways was considerably

reduced in DP1-deficient mice compared with wild-type animals. Levels of T_H2 cytokines (that is, IL-4, IL-5 and IL-13) in the BAL fluid recovered from DP1-deficient mice following OVA challenge were considerably lower than the levels of these cytokines in the BAL fluid of wild-type mice, whereas no difference in levels of the T_H1 cytokine IFN-γ was noted, indicating a specific reduction in the recruitment of T_H2 cells. Consistent with a reduction in airway lymphocytes and T_H2 cytokines, DP1-deficient mice showed decreased airway eosinophil recruitment, mucus hypersecretion and AHR. The concentrations of both total IgE and OVA-specific IgE were markedly increased in response to OVA immunization and were boosted by subsequent inhalation of OVA in a similar manner in both wild-type and DP1-deficient mice. These observations indicate that PGD_2, produced by Fc receptor crosslinking of airway mast cells and/or alveolar macrophages in response to allergic challenge, binds to DP1 in the lungs to induce the recruitment of lymphocytes to the site of challenge. Similar to BLT1-deficient mice, the defect in lymphocyte trafficking in DP1-deficient mice could be overcome by increasing the intensity of allergen challenge[85], indicating that PGD_2 and LTB_4 might have important roles in the initiation of asthma or asthma exacerbations but might not be required for the persistence of established disease.

The ability of PGD_2 to mediate T-cell trafficking in allergic pulmonary inflammation was further supported by the generation of transgenic mice that overexpress PGDS in various organs[86], including the lungs[87]. In an active immunization asthma model, PGD_2 levels in the lungs were considerably higher in *Pgds*-transgenic mice than in wild-type animals, and this increase in PGD_2 production was associated with increased airway lymphocyte recruitment[87]. Furthermore, the levels of IL-4 and IL-5 in the BAL fluid following inhaled allergen challenge were markedly higher in *Pgds*-transgenic mice than in wild-type animals, whereas the concentration of IFN-γ was considerably lower, indicating that the increased airway lymphocyte recruitment in *Pgds*-transgenic mice specifically included T_H2 cells. Consistent with increases in airway lymphocytes and T_H2 cytokines, *Pgds*-transgenic mice showed increased airway eosinophil recruitment. Interestingly, the percentage increase in airway lymphocyte recruitment in *Pgds*-transgenic animals relative to wild-type mice was greater after one challenge with OVA than after three challenges[87], further indicating that PGD_2-directed T_H2-cell trafficking is important early after the initiation of allergic pulmonary inflammation.

The mechanism by which PGD_2 mediates T_H2-cell recruitment to the airways was elucidated in an investigation of the effects of exogenous PGD_2 on allergic pulmonary inflammation[88]. Pretreatment of OVA-immunized mice with aerosolized PGD_2 caused a marked increase in T_H2-cell recruitment to the airways after one aerosol challenge with a dose of OVA that by itself was low enough to induce only minimal T_H2-cell responses. Consistent with this, PGD_2 pretreatment caused increases in BAL levels of T_H2 cytokines and numbers of eosinophils. By contrast, in the absence of subsequent antigen challenge, aerosolized PGD_2 induced no recruitment of T_H2 cells to the airways of immunized mice, despite the fact that T_H2 cells express DP2. This finding indicates that the PGD_2 augmentation of T_H2-cell recruitment in this model results from PGD_2-induced production of other T_H2-cell chemoattractants by resident cells of the lungs, rather than from PGD_2 itself directly functioning as a T_H2-cell chemoattractant. Expression of DP1 is upregulated by lung epithelial cells following aeroallergen challenge[85], indicating that airway epithelial cells might be the source of PGD_2-induced T_H2-cell chemoattractants.

The chemokine CCL22 (also known as MDC) is produced by airway epithelial cells and has been implicated in allergic pulmonary inflammation[89,90]; and its cognate receptor, CCR4, is expressed by T_H2 cells[30–32]. PGD_2 induces CCL22 expression by primary human bronchial epithelial cells *in vitro*, and pretreatment of immunized mice with PGD_2 induced increased expression of CCL22 by bronchial epithelial cells after a low-dose OVA challenge *in vivo*[88]. Injection of neutralizing antibody specific for CCL22 inhibited most of the T_H2-cell recruitment to the airways of PGD_2-pretreated mice challenged with a low-dose of OVA, indicating that PGD_2-induced CCL22 expression was mostly responsible for the augmentation of T_H2-cell airway recruitment by PGD_2 in these experiments. Consistent with its inhibition of T_H2-cell recruitment, antibody specific for CCL22 also considerably reduced the airway levels of IL-4 and IL-5, the number of eosinophils and AHR in PGD_2-pretreated OVA-challenged mice[88].

As noted earlier, immunized BLT1-deficient, DP1-deficient and mast-cell-deficient mice all showed defects in airway lymphocyte recruitment after limited aeroallergen challenge, which is also the time point at which the *Pgds*-transgenic mice showed the greatest relative increase in airway lymphocyte recruitment. Taken together, these results support the hypothesis that mast-cell (and possibly alveolar macrophage) activation by antigen-induced Fc receptor crosslinking directs T-cell recruitment to the airways early after antigen challenge, through the generation of the lipid mediators LTB_4 and PGD_2. However, the defects in lymphocyte trafficking in the BLT1-deficient, DP1-deficient and mast-cell-deficient mice could be overcome by repeated-dose or higher-dose airway antigen exposures, indicating that chemoattractants other than LTB_4 and PGD_2, generated later after antigen challenge, are sufficient for airway T-cell recruitment during the amplification and/or maintenance of allergic pulmonary inflammation.

Chemokines direct T-cell trafficking

Evidence indicates that T-cell recruitment during the amplification and/or maintenance of allergic pulmonary inflammation is directed by peptide chemokines. T_H2 cells preferentially express the chemokine receptors CCR3, CCR4 and CCR8 (REFS 30–32), and the levels of the chemokines that bind to these receptors have been noted to be increased in allergic inflammation. In models of

asthma, T_H2-cell recruitment has not been abrogated by inhibition of gene expression or deletion of the genes encoding single chemokines that attract T_H2 cells, such as CCL11 (also known as eotaxin-1), CCL22 or CCL1 (also known as TCA3), or individual T_H2-cell chemokine receptors, including CCR3, CCR4 or CCR8 (REFS 91–96); this indicates that multiple chemokines control T_H2-cell trafficking in this phase of allergic pulmonary inflammation. By contrast, a global deficiency of chemokines that attract T_H2 cells, as observed in mice deficient for STAT6 (signal transducer and activator of transcription factor 6) resulted in a marked reduction of T_H2-cell trafficking in an adoptive-transfer model of asthma[97]. After seven aerosol challenges with OVA, the number of transferred T_H2 cells recruited to the airways was markedly decreased in the STAT6-deficient recipients compared with wild-type animals, as were all other components of the asthma phenotype, including eosinophil recruitment, mucus hypersecretion and AHR. Consistent with these results, chemokines that attract T_H2 cells — including the CCR3 agonists CCL11 and CCL24 (also known as eotaxin-2), the CCR4 agonists CCL22 and CCL17 (also known as TARC) and the CCR8 agonist CCL1 — were all noted to be diminished in the STAT6-deficient recipients, indicating that T_H2 cytokines induce chemokine production by resident cells of the lungs by signalling through STAT6. In support of this conclusion, IL-4 and IL-13 have been noted to induce the expression of CCL11, CCL24 and CCL26 (also known as eotaxin-3) by airway epithelial cells and endothelial cells *in vitro* and *in vivo*[98–102], and IL-4 induction of CCL11 expression was found to be STAT6 dependent[103]. IL-4 has also been shown to induce the expression of CCL22 and CCL17 by macrophages and respiratory epithelial cells[92,104].

Cooperation of eicosanoids and chemokines

The experimental evidence obtained from the mouse models of asthma that we have discussed indicates non-redundant roles for LTB_4, PGD_2 and chemokines in the recruitment of T cells to the airways in allergic pulmonary inflammation, as shown in FIG. 5. In individuals with air-borne-antigen-specific IgE that has been generated as a result of previous exposure to antigen, recurrent exposure to the same antigen leads to activation of airway mast cells and alveolar macrophages through Fc receptor crosslinking, which in turn leads to the generation of LTB_4 and PGD_2 in the airways. The LTB_4 produced can direct the earliest phase of airway T-cell recruitment through BLT1 expressed by effector T cells. The PGD_2 generated might similarly participate in this earliest phase of T-cell recruitment through DP2 expressed by effector T_H2 cells, although determining whether PGD_2 directly recruits T cells through DP2 immediately after mast-cell and alveolar macrophage activation awaits the generation of mice that are genetically deficient in this receptor. In either case, PGD_2 participates in the next phase of airway T-cell recruitment following allergen exposure by inducing the expression of CCL22 in the airways through DP1 expressed by respiratory epithelial cells. After early eicosanoid-directed T-cell recruitment, T_H2 cytokines produced by these T_H2 cells that are recruited early initiate the next phase of T-cell recruitment, by inducing the STAT6-dependent expression of T_H2-cell chemotactic chemokines by resident lung cells. This chemokine-directed T-cell recruitment can then amplify and maintain allergic pulmonary inflammation.

Clinical evidence from IgE-specific monoclonal antibody therapy indicates that the eicosanoid-directed T-cell recruitment that is involved in the initiation of allergic pulmonary inflammation in mouse models might also contribute to the clinical manifestations of asthma in patients with established disease, possibly in the initiation of clinical exacerbations. A humanized mouse monoclonal antibody specific for the FcεRI-binding domain of human IgE has been shown to inhibit the binding of IgE to mast cells without provoking mast-cell activation[105]. Treatment of patients with this IgE-specific monoclonal antibody (known as omalizumab) decreased air-flow obstruction provoked by allergen challenge during both the early and late phases of the asthmatic response[106] (TABLE 3). The airway response to aeroallergens in asthmatic patients is typically biphasic: a transient early air-flow obstruction occurs within minutes of challenge and is associated with IgE-dependent mast-cell activation, and a later phase of air-flow obstruction occurs several hours after challenge and is thought to be mediated by T_H2 cells[2,107]. The role of IgE in the late-phase response is uncertain, but mitigation of the late-phase response using IgE-specific antibody therapy indicates that IgE-dependent mast-cell activation might participate in its pathogenesis. Evidence from mouse models of asthma that the early recruitment of T_H2 cells to the airways following allergen exposure is directed by mast-cell-derived LTB_4 and PGD_2 identifies a mechanism through which mast-cell activation could contribute to late-phase asthmatic responses in humans.

Consistent with its effects on asthmatic responses to allergen challenge, in patients with moderate to severe asthma, IgE-specific antibody treatment has been shown to reduce the frequency of asthma exacerbations — the primary end-point in clinical trials of this therapy[108,109]. The inflammatory mechanisms that are responsible for clinical exacerbations of chronic asthma are not yet understood. At the time of hospitalization for exacerbations, plasma levels of LTB_4 have been noted to be considerably increased in patients with asthma compared with LTB_4 levels in the same patients when asthma is controlled[46]. Similarly, urinary levels of 9α,11β-PGF_2, an initial metabolite of PGD_2, were observed to be increased on the first day of exacerbations in asthmatic children and then to decrease in serial measurements as symptoms resolved during hospital treatment[110]. These increases in the levels of LTB_4 and PGD_2 indicate that T-cell recruitment by these eicosanoids might contribute to the initiation of clinical asthma exacerbations, in a manner analogous to the induction of T-cell recruitment by these eicosanoids in the initiation of allergic pulmonary inflammation in mouse models of asthma. If so, reduced levels of LTB_4

and PGD_2, as would be expected to result from decreased mast-cell activation, might contribute to the decrease in exacerbation frequency that is associated with IgE-specific antibody therapy.

This hypothesis raises the possibility that LTB_4, PGD_2 and/or their cognate receptors might be good targets for new asthma therapies (TABLE 3). Zileuton — a 5-LO inhibitor that blocks the production of both LTB_4 and the cysteinyl leukotrienes — has been shown to improve disease control in patients with mild to moderate asthma[111], and antagonists that specifically target BLT1 have shown promise in animal models of asthma. A selective LTB_4 receptor antagonist, known as CP-105,696, completely abrogated the increased AHR induced in a primate model of asthma[112], although the long half-life of this antagonist in humans has precluded its clinical use[113]. Another LTB_4 receptor antagonist, known as BIIL 284 (REF. 114), is currently being

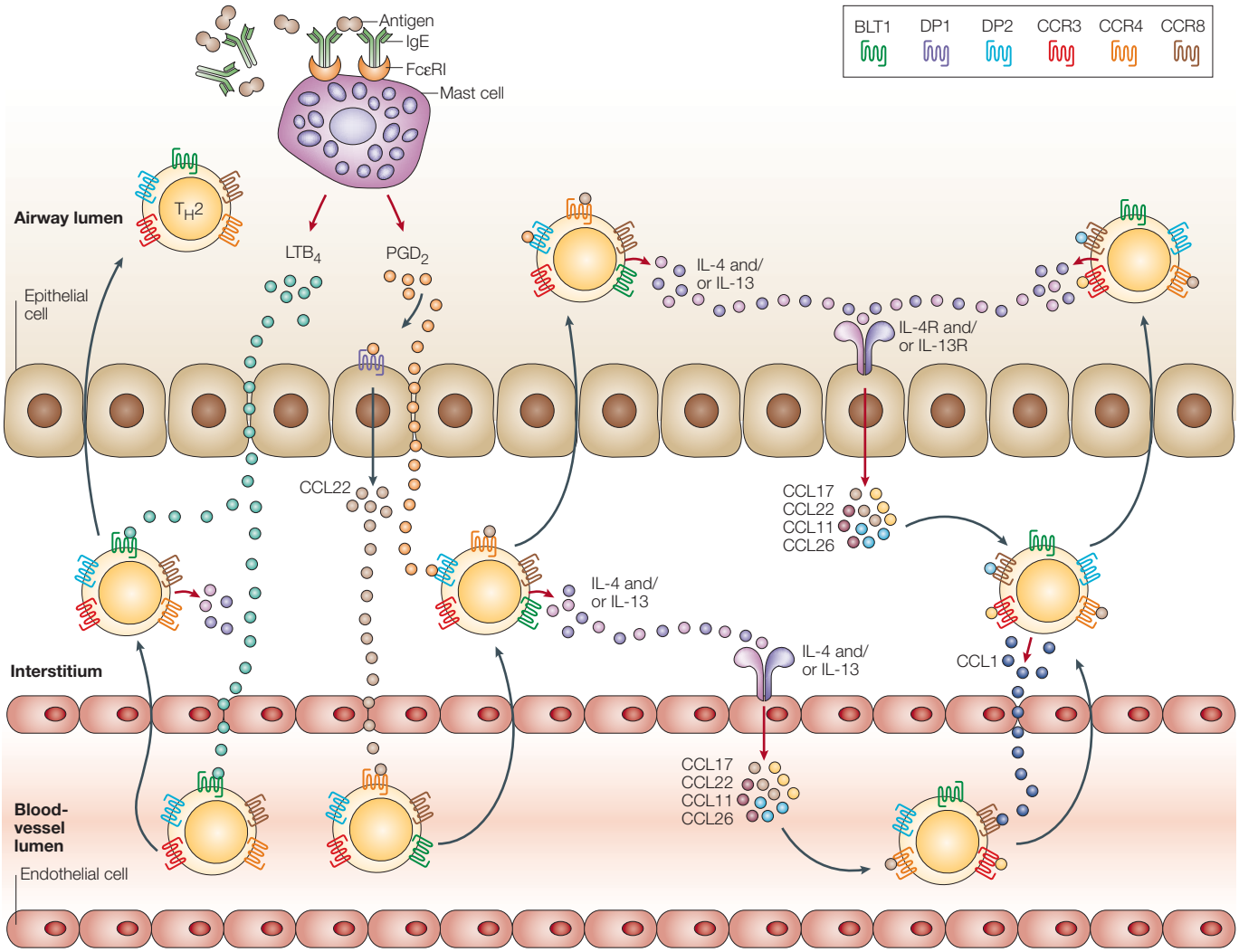

Figure 5 | **Three waves of T-cell chemoattractants in asthma: LTB_4, PGD_2 and chemokines.** Experimental evidence from mouse models of asthma indicates that leukotriene B4 (LTB_4), prostaglandin D_2 (PGD_2) and chemokines have non-redundant, cooperative roles in directing the recruitment of T cells to the airways in allergic pulmonary inflammation. When sensitized individuals are exposed to environmental antigens, LTB_4 and PGD_2 are generated in the airways by mast cells (shown) and alveolar macrophages, which are activated through Fc receptor crosslinking. The LTB_4 that is generated can direct the earliest phase of airway T-cell recruitment by interacting with BLT1 that is expressed by effector T cells. The PGD_2 that is generated might also participate in this earliest phase of T-cell recruitment by interacting with DP2 that is expressed by effector T helper 2 (T_H2) cells, but this has not yet been experimentally shown. PGD_2 has been shown to participate in the second phase of airway T-cell recruitment, by inducing respiratory epithelial-cell expression of CC-chemokine ligand 22 (CCL22) through its interaction with DP1, which is expressed by these epithelial cells. CCL22 recruits T cells through binding to its cognate receptor CC-chemokine receptor 4 (CCR4), which is expressed by T_H2 cells. After this early T-cell recruitment directed by LTB_4 and PGD_2, production of interleukin-4 (IL-4) and IL-13 by recruited T_H2 cells initiates a third phase of T-cell recruitment. These T_H2 cytokines induce resident lung cells, such as epithelial cells, endothelial cells, fibroblasts and macrophages, to express several chemokines that can attract T_H2 cells. Ligands of T_H2-cell expressed chemokine receptors include the CCR3 agonists CCL11 and CCL24, the CCR4 agonists CCL22 and CCL17, and the CCR8 agonist CCL1. CCL1 might also be produced by the recruited T cells. The additional T cells that are directed to the airways in response to these chemokines can then amplify and maintain allergic pulmonary inflammation. Red arrows indicate secretion of chemoattractants. Black arrows indicate migration of T_H2 cells. FcεRI, high-affinity receptor for IgE; IL-4R, interleukin-4 receptor.

Table 3 | **Agents affecting eicosanoids in asthma**

Therapy	Effector function	Species/model	Therapeutic Effect	References
IgE-specific monoclonal antibody (Omalizumab)	Inhibits IgE binding to, and activation of, mast cells	Human patients with moderate to severe asthma	Decreases air-flow obstruction, reduces frequency of asthma exacerbations	106, 108,109
Zileuton	Inhibits 5-LO, so blocks the production of LTB_4 and cysteinyl leukotrienes	Human patients with mild to moderate asthma	Improves disease control	111
CP-105,696	Antagonist of LTB_4 receptor	Primate model of asthma	Abolishes airway hyper-responsiveness	112
Aspirin	Inhibits COX1 and COX2, so blocks the synthesis of prostaglandins, including PGE_2 and PGD_2	Subset (~5–20%) of human patients with asthma	Exacerbates asthma	115
S-5751	Antagonist of DP1	Guinea-pig model of asthma	Inhibits allergic pulmonary inflammation	116
Ramatroban (BAY u3405)	Antagonist of DP2 and TXA_2 receptor	Human patients with asthma	Reduces bronchial hyper-responsiveness	117,118

5-LO, 5-lipoxygenase; COX, cyclooxygenase; LTB_4, leukotriene B4; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; TXA_2, thromboxane A_2.

assessed for safety and efficacy in patients with cystic fibrosis. Agents that block prostaglandin synthesis by the inhibition of COX can actually exacerbate asthma, an effect thought to be caused by inhibition of COX1-dependent synthesis of PGE_2, which is an important bronchodilator and an endogenous inhibitor of leukotriene production and histamine release[115]. However, agents that specifically inhibit PGD_2 synthesis or block its receptors might have therapeutic potential. S-5751 — a DP1 receptor antagonist — markedly inhibited allergic pulmonary inflammation in a guinea-pig model of asthma[116]; and ramatroban (also known as BAY u3405) — a dual antagonist of DP2 and the thromboxane receptor TXA_2 (REF. 117) — reduced bronchial hyper-responsiveness in asthmatic patients[118].

Concluding remarks

In addition to their classically described roles in asthma, recent experimental evidence now indicates that LTB_4 and PGD_2 cooperate with chemokines such that they have non-redundant, sequential roles in directing the recruitment of T cells to the airways in allergic pulmonary inflammation. In mouse models of asthma, the lipid mediators LTB_4 and PGD_2 have crucial roles in directing T cells to the airways immediately after the initiation of pulmonary allergic inflammation, 'greasing the way' for the chemokine-mediated recruitment of more T cells to the airways during the amplification and maintenance of the asthma phenotype. The increased levels of LTB_4 and PGD_2 that have been noted in patients with asthma exacerbations, together with the ability of IgE-specific antibody therapy to decrease the frequency of exacerbations, indicate that T-cell recruitment by mast-cell-derived LTB_4 and PGD_2 might also contribute to the initiation of clinical exacerbations in patients with asthma — although definitively establishing this will require further studies. If the initiation of patient exacerbations is similar to the initiation of allergic pulmonary inflammation in mouse models, then these lipid mediators and/or their receptors could be attractive targets for additional asthma therapies.

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