On Naivity of T Cells in Inflammatory Bowel Disease: A Review

Carolijn Smids, MD,* Carmen S. Horjus Talabar Horje, MD,† Peter J. Wahab, MD, PhD,* Marcel J. M. Groenen, MD, PhD,* Sabine Middendorp, PhD,‡ and Ellen G. van Lochem, PhD‡

Abstract: Little is known about different phases of T-cell maturation in gut mucosa. Based on current knowledge about the migratory pathways of naive and memory T cells, it is believed that access to peripheral, nonlymphoid tissues is restricted to memory T cells. Surprisingly, there is increasing evidence of high numbers of naive T cells in the chronically inflamed gut tissue of patients with inflammatory bowel disease. This could partially be explained by new formation of ectopic lymphoid organs. Ongoing recruitment of naive T cells at inflammatory sites might play a role in the immunopathogenesis of inflammatory bowel disease.

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Inflammatory bowel diseases (IBDs) are believed to result from aberrant inflammatory responses to the intestinal bacterial antigens in a genetically susceptible individual. Available evidence argues an important role for the adaptive immune system in IBD. Most research has focused on increased migration and proliferation of activated or memory T cells (Th1, Th2, Th17, and regulatory T cells) in the inflamed gut mucosa.1,2 As naive T cells (T_N) are normally thought to be excluded from inflamed tissue, the maturation process at extralymphoid effector sites, like the inflamed gut mucosa in IBD, has received less attention. However, T_N are the initiators of the inflammatory cascade of activated T-cell expansion and differentiation and may therefore play a central role in inflammatory processes.

Lymphoid progenitors originating from bone marrow stem cells undergo maturation and selection in the thymus to become functional CD4+CD8− or CD4−CD8+ (single positive) T lymphocytes. In this thymic selection process, T cells that can recognize peptide in the context of self-major histocompatibility complex molecules are positively selected, whereas autoreactive T cells are deleted in the thymus.3 Of all T cells entering the thymus, the stringent thymic selection leaves only 3%.4 The T_N cells leaving the thymus still have to experience antigen-driven post-thymic maturation before they proliferate and differentiate into memory T cells.5

The classical view states that T_N cells exclusively recirculate between secondary lymphoid organs (SLOs) (e.g., lymph nodes, Peyer’s patches [PPs], and the spleen) through lymph and blood, whereas only memory T cells are able to enter nonlymphoid organs.6 However, recent evidence has shown that T_N cells routinely traffic through nonlymphoid organs and might be activated outside the confined lymphoid complex.7–16 These findings shed new light on the role of T_N cells in autoimmune disease.

This review summarizes the current knowledge of the involvement of T_N cells in IBD. We focus on the migration and activation of these cells in the nonlymphoid, healthy, and inflamed gut.

T-CELL SUBSETS: MATURATION AND ACTIVATION

Human T cells can be subdivided into different sets, either according to their immunophenotype (expression of leukocyte differentiation markers) or functionality (production of chemokines). Considering phenotypic characterization, the 2 main categories are T_N cells, which have not yet encountered any (foreign) antigen, and memory T cells, which are antigen experienced. The phenotypic changes during the antigen-driven maturation and activation process are depicted in Table 1 and Figure 1.

T_N cells can be subdivided into 2 subsets: recent thymic emigrants (RTEs), also called “true naive,” and mature naive T cells (T_MN).7 RTEs have not yet met the antigen for their specific T-cell receptor and have no proliferation history. T_MN cells are also “antigen inexperienced” but underwent proliferation in response to homeostatic survival signals.17 RTEs can be identified by the expression of CD45RA, CD31, CD27, CD28, CCR7, and CD62L18 and are enriched with T-cell receptor excision circles (TRECs).4 TRECs are nonreplicative circular excision DNA products of the T-cell receptor gene rearrangement.17,18 Post-thymic proliferation of RTEs causes diminishing of TRECs and downregulation of a set of surface markers (CD31 and PTK7), generating “proliferative-experienced” T_MN cells.4
T cells that have encountered antigen can immunophenotypically be subdivided into central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and effector memory T cells re-expressing CD45RA (T_{EMRA}). During maturation, T_{CM} express CD45RO instead of CD45RA but retain CCR7 and CD62L, which are important homing markers for lymphoid tissue. During further maturation, T_{EM} cells downregulate both CCR7 and CD62L and acquire new adhesion markers, such as the gut-homing markers α4β7 and CCR9.20–22 A subset of effector memory T cells, known as T_{EMRA} cells, (re)expresses CD45RA, and occasionally also CCR7, but remains distinguishable from T_{N} cells by their lack of CD27, CD28, and CD62L. When defining T_{N}-cell subsets by immunophenotype, the use of at least 2 markers is recommended.18 Different combinations have been suggested: CD45RA and CD27/CD2818 or CCR7 and CD27.23 Unfortunately, this is not yet a common practice, and T cells are often defined as naive or memory solely by the expression of CD45RA/RO. This makes the distinction between T_{N} cells and T_{EMRA} cells practically impossible. Contamination of the T_{N}-cell subset with T_{EMRA} cells may lead to inaccurate conclusions and hampers comparability between studies.

### TABLE 1. Different Maturation Stages of Human T Cells Are Distinguished by Various Cell Surface Markers

<table>
<thead>
<tr>
<th>Surface Markers</th>
<th>RTE</th>
<th>T_{MN}</th>
<th>T_{CM}</th>
<th>T_{EM}</th>
<th>T_{EMRA}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD31</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CD45RA</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CCR7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>±</td>
</tr>
<tr>
<td>CD62L</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CD27</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>−</td>
</tr>
<tr>
<td>CD28</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>−</td>
</tr>
<tr>
<td>CD45RO</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Normal expression (+), moderate expression (±), and no expression (−).

CCR7, chemokine receptor 7; CD, cluster of differentiation; and CD62L, L-selectin.

### MIGRATION OF NAIVE T LYMPHOCYTES DURING HOMEOSTASIS

Migration of immune cells is a process referred to as homing. The general belief before 1964 was that lymphocytes randomly circulate throughout the body. The first signs hinting otherwise were found in rats and mice,24,25 showing that certain lymphocytes only homed to the lymph node or the spleen. Thirty years later, these findings were confirmed in sheep. Additionally, distinct migratory routes for T_{N} and memory T cells were suggested.6 Most of our further understanding on T-cell migration and proliferation is still based on research in animal models, although it is obvious that these models (with a lifespan of several months up to a few years in pathogen-free conditions) cannot reflect the human exposure to multiple pathogens over decades.26 Human studies are still limited because only peripheral blood or tissues from surgical resection material are easily accessible.

The formerly accepted migration model with distinct routes for T_{N} and memory T cells turns out not to be as segregated as suggested before. T_{N} cells not only circulate through blood and lymph between SLOs but also enter nonlymphoid organs, which were first thought to be exclusively confined to memory T cells. T_{N} cells have recently been identified in nonlymphoid tissue of healthy individuals, representing approximately 10% of the T cells at the intestinal sites (jejunum, ileum, and colon).14 Additionally, up to 50% of the T cells in nonlymphoid organs of the gut in steady-state mice were shown to express CCR7 (T_{N}, T_{CM}, and T_{EMRA}).10 An immune surveillance or tolerance induction role of T_{N} cells entering the nonlymphoid tissues has been advocated.11 Although central tolerance during thymic selection eliminates most of the autoreactive T cells, some of them might escape and migrate to the periphery. In this way, naive autoreactive T cells (RTEs and T_{MN}) could enter healthy tissues, become

![CD45RA, CD45RO, CCR7, CD27, CD28, CD31](image_1)

**FIGURE 1. Human T-cell maturation subsets. Immunophenotypical subsets of T cells by expression of several cell surface markers. Intensity of color of markers indicates level of expression. CCR7, chemokine receptor 7; CD62L, L-selectin.**
activated, and initiate chronic inflammation. However, influx of T<sub>N</sub> cells in nonlymphoid tissue possibly induces tolerance to organ-specific antigens within the tissue itself.<sup>17</sup> Antigen presentation to T<sub>N</sub> cells takes place primarily in the SLOs. After antigen recognition, further maturation of T<sub>N</sub> cells evolves toward memory T cells, which express tissue-specific surface receptors. T<sub>CM</sub> cells express the same lymph node homing molecules as T<sub>N</sub> cells, namely, L-selectin (CD62L) and chemokine receptor 7 (CCR7, also known as CD197), and therefore also have the potential to home to SLOs.<sup>27</sup> T<sub>EM</sub> cells are CCR7−CD62L− and express tissue homing molecules like α4β7, which binds to mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and CCR9, which interacts with chemokine (C-C motif) ligand 25 (CCL25) in the gut endothelium.<sup>12</sup> T<sub>N</sub> cells usually do not express tissue-specific homing receptors on their surface, with an exception of moderate levels of α<sub>4</sub>β<sub>7</sub>. They enter the lymph node and PPs across specialized postcapillary venules called high endothelial venules (HEVs). HEV endothelial cells are cuboidal and are surrounded by multiple layers of fibroblastic reticular cells that produce various extracellular matrix components, forming a thick basal lamina. Microscopically, they can easily be distinguished from normal venules, which have flat endothelium and a thin basal lamina.<sup>28</sup> HEV endothelium expresses molecules that function in lymphocyte trafficking. This occurs through interaction between surface molecules on the lymphocytes with their HEV-expressing ligand counterparts: CD62L on the T cells with peripheral lymph node addressin (PNAd) and MAdCAM-1, CCR7 on the T cell with CCL21, and lymphocyte function-associated antigen 1 (LFA-1, also known as CD11aCD18) on the T cell with intercellular adhesion molecule 1 (ICAM-1, also known as CD54) (Fig. 2A).<sup>12, 29</sup> In contrast to HEV in peripheral lymph nodes, HEV in PPs do not express PNAd. In PPs, the tethering/rolling step is mediated by the interaction of CD62L and α4β7 with MAdCAM, whereas further adhesion and migration of T cells happen through LFA-1 and ICAM-1 (Fig. 2B).<sup>30</sup> The binding of CD62L to MAdCAM-1 is dependent on the presence of specific carbohydrate ligands on the MAdCAM-1.<sup>31, 32</sup> Evidence supporting this interaction is demonstrated in mice studies but is still missing for human.

**MIGRATION OF NAIVE T LYMPHOCYTES IN IBD**

**Tertiary Lymphoid Organs**

There is increasing evidence for primary T-cell responses independent of SLOs. Chronic inflammation and solid tumors have been found to induce formation of HEV-like vessels in nonlymphoid inflamed tissue and tumor tissue, organizing around so called tertiary lymphoid organs (TLOs).<sup>33–37</sup> These TLOs are ectopic accumulations of lymphoid cells, arising at a nonlymphoid

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**FIGURE 2.** Migration of T<sub>MN</sub> and T<sub>CM</sub> during homeostasis. A, T<sub>MN</sub> and T<sub>CM</sub> cells migrate into a lymph node by interaction with surface receptors on HEV. CD62L binds with PNAd (1), chemokine receptor 7 (CCR7) binds with chemokine (C-C motif) ligand 21 (CCL21) (2), and LFA-1 binds with intercellular adhesion molecule 1 (ICAM-1) (3). B, T<sub>MN</sub> and T<sub>CM</sub> cells migrate into PPs by interaction with surface receptors on HEV. Tethering and rolling is mediated by interaction of mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and CD62L (1a) and α4β7 integrin (1b). Further adhesion and migration occur through CCR7 with CCL21 (2) and LFA-1 with ICAM-1 (3).
location through a process called lymphoid neogenesis. Contrarily, primary lymphoid organs and SLOs are found at specific, predefined sites that seem to be genetically fixed and develop before birth.38 TLOs morphologically resemble SLOs because they also have T- and B-cell compartments, lymphoid chemokines, antigen-presenting cells (like dendritic cells), and HEV-like vessels and are therefore also able to recruit TN cells. 

How and why TLO formation occurs is unclear. HEV-like vessels presumably facilitate lymphocyte influx into TLOs but it is also possible that TN cells gain initial access to nonlymphoid tissues through regular blood vessels and that HEVs are formed later in the process.8 Lymphotoxin α1β2 (LTα1β2, a tumor necrosis factor family member) may have a prominent role in regulating the formation of (secondary and tertiary) lymphoid organs and is crucial for HEV development and maintenance.29,40 After priming, TN CD4+ cells induce LTα1β2 expression on their surface.41,42 The presence of ectopic CCL21 on blood vessels during inflammation may trigger the influx of CCR7+ T cells (TN and TCM). This provides a source of LTα1β2, perpetuating CCL21 expression and facilitating the formation of HEV-like vessels and TLOs. Notably, blocking the lymphotoxin pathway in colitis mouse models reduced the severity of inflammation and was equally efficacious as anti–tumor necrosis factor α therapy.43,44

Clinical significance of TLOs has already been described in other diseases like colorectal cancer and rheumatoid arthritis. In early-stage colorectal cancer, TLOs are associated with a better prognosis45 and are suggested as a possible marker for prognosis.46 In patients with rheumatoid arthritis, TLOs were present in 49% of synovial tissue, and its presence coincided with longer disease duration and lower response to therapy (more refractory disease).47 Reversal of TLOs was a good marker of response to therapy.

Tertiary Lymphoid Organs in IBD

Recent studies have shown that TLOs might play a role in chronic inflammation of the gut in IBD. Ectopic CCL21 on blood vessels and PNAd-expressing HEV-like vessels have been detected in inflamed gut tissue of patients with ulcerative colitis (UC). A positive correlation between CCL21 expression and influx of TN cells was observed.8 CCL21 production was demonstrated in inflamed tissue of patients with Crohn’s disease (CD) and UC, and lymphoid aggregates were found in 50% of their intestinal biopsy samples.35 HEV-like vessels in the active phase of UC and CD were identified only in inflamed gut tissue compared with a scarce presence of these vessels in noninflamed colon or during remission of IBD.58,49 Furthermore, the formation of ectopic lymphoid follicles or TLOs was found in diseased ileal segments of patients with CD.50 In contrast to the chronically inflamed IBd tissue, acute inflammation was not accompanied by the induction of lymphoid aggregates and HEV-like vessels.48 MAdCAM-1+ intestinal lamina propria venules are increased in UC, both in active disease and remission, compared with normal controls.51 There was great coexpression of PNAd among MAdCAM-1+ vessels in patients with active disease (66.5%) when compared with patients in remission (7.62%), and PNAd was not detected in the colonic mucosa of normal controls. In a recent study in mice with chronic ileitis, the induction of ectopic lymphoid tissue has been demonstrated in the inflamed intestine, with a statistically significant increase of CCL21 expression and accumulation of TN cells, TEM, and TCM cells in the terminal ileum compared with healthy wild-type mice.15

Thus far, it remains difficult to distinguish gut-associated lymphoid tissue from TLOs and an unambiguous definition is lacking.33 Nonetheless, chronically inflamed gut induces organized structures that cannot be observed in the gut of healthy controls. These structures, referred to as TLOs, are present in the deeper layers of the epithelium and contain PNAd-expressing HEV-like vessels that distinguish them from the submucosal PPs.48,50,51

NAIVE T CELLS IN IBD

Mice: The T-Cell Transfer Model

Immunodeficient mice (CB17 severely combined immunodeficient/recombinase-activating gene 2-/-) develop chronic, severe intestinal inflammation after transfer of naive (CD4+CD45RBhigh) donor T cells into their peritoneal cavity.52 Cotransferring memory (CD4+CD45RBlow) T cells inhibits the development of intestinal inflammation,53 and single transfer of the memory subset does not cause inflammation.54 One could expect that the transferred TN cells are primed within gut-associated lymphoid tissue and mesenteric lymph nodes before inducing colitis. However, the induction of colitis by transfer of TN cells was also demonstrated in mice that are deficient of SLOs (gut-associated lymphoid tissue, mesenteric lymph node, and spleen).55 This study underlines that SLOs are not required for priming TN cells into colitogenic effector T cells to initiate colitis, leaving the site of priming in these models to be defined. As the pattern of exacerbation and remission of human IBD has not been perfectly phenocopied in these mouse models, the presence and the role of TN cells in the gut of IBD remain to be elucidated.56

The Potential Role of Naive T Cells in Human IBD

The first suggestions indicating a potential role of (maturation of) TN cells in the pathogenesis of IBD came from a few (case) reports showing the effectiveness of thymectomy in adult patients with IBD.57-60 Thymectomy has been performed in patients with UC with thymic hyperplasia and was successful for maintaining remission in patients refractory to conventional therapy.58,60 Additionally, thymectomy was also performed in a patient with CD developing myasthenia gravis, leading to remission of her CD activity.59 No further publications have arisen on the potential of thymectomy in IBD.

Already, in 1995, it was revealed that chronic inflammation in CD induced migration of TN cells to inflamed sites. The study involved ileal samples of patients with CD and bowel samples of controls with colon carcinoma. In the intestines of controls, blood vessels were only found in the lamina propria and beneath the muscularis mucosa, and they exhibited the characteristics of
normal (flat) venules. The inflamed intestines of patients with CD showed numerous dilated and highly activated small vessels with HEV characteristics, extending from the superficial mucosa layers throughout the deep submucosa. These vessels and the perivascular and mucosal infiltrates all harbored mainly macrophages and T cells. The majority of T cells within the (sub)mucosal infiltrates were T_N (CD45RA+CD31+CD62L+CD27+) and T_CM (CD45RO+CD31−CD62L−CD27−) cells. The normal intestinal mucosa of controls was predominantly populated by T_EM cells (CD45RO−CD31−CD62L−CD27−).7

The expression of maturation markers on CD4+ T cells was analyzed in the intestinal mucosa of both patients with CD and UC with active disease. Almost all patients were using corticosteroid medication and were included at the time they underwent partial resection of the intestine. Noninvolved bowel segments of patients undergoing bowel resection for cancer treatment were used as control. The numbers of CD45RA+ T cells were increased in the intestine of patients with IBD (49.0% in UC and 27.7% in CD) as compared with controls (4.5%). The CD45RA+ cells found in patients with IBD were mainly located in “follicular structures.”9

Another study aimed to characterize T cells present in the intestine of patients with IBD before and after surgery. Sigmoid-derived biopsy specimens of patients with IBD (CD and UC) with distal active colitis were evaluated. Patients who were in remission with mucosal healing showed a reduction of >50% of CD4+CD45RA+ T cells compared with inflamed mucosa of patients with active disease. There was no difference in the amount of memory T cells in the inflamed mucosa when compared with noninflamed mucosa.16

Regarding RTEs in patients with IBD, increased TREC levels in mucosal but not peripheral blood lymphocytes were found in patients with UC, indicating direct recruitment of RTEs into the inflamed mucosa.18 However, these results have not yet been duplicated.

We recently studied the maturation of T cells in the gut mucosa in a large group of newly diagnosed, untreated patients with IBD. Remarkably, high frequencies of T_N were identified in a subgroup of patients with CD and UC (naive profile). This group of patients expressed different proinflammatory cytokine levels (higher tumor necrosis factor α and lower interferon γ) compared with the group of patients with high frequencies of T_EM. Furthermore, there was an association between the naive profile and extended disease location in CD.51 We elaborated on the possible correlation between differences in the maturation of the T-cell subsets early in disease course and disease heterogeneity. An extended follow-up of this cohort may elucidate the prognostic value of different maturation profiles at primary diagnosis.

CONCLUSIONS
Contrarily to previous ideas, there is increased evidence that T_N cells migrate to nonlymphoid organs in homeostasis and even more in chronic inflammation. During chronic inflammation, migration of T_N cells could be facilitated by TLOs. Formation of these TLOs seems dependent on LTAβ1β2; however, the signals triggering these mechanisms are waiting to be characterized. Presence of TLOs implies an ongoing recruitment of T_N and T_CM cells to the gut mucosa, maintaining a continuous inflammatory process. The relevance of targeted therapy against this phenomenon has not yet been studied. It is conceivable that a process involving dissolution of TLOs could restore immunologic homeostasis.

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