ILC3 in Axial Spondyloarthritis: the Gut Angle

Daniele Mauro [1,2] · Federica Macaluso [1,2] · Serena Fasano [1] · Riccardo Alessandro [3] · Francesco Ciccia [1]

Abstract

Purpose of Review A growing body of evidence supports the relevance of the interleukin-23/interleukin-17 (IL-23/IL-17) pathway for the pathogenesis of axial spondyloarthritis (axSpA) and its treatment. Recently, innate lymphoid cells (ILC), a heterogeneous family of immune effector cells, have been identified as a relevant contributor in tissue homeostasis, partially via IL-23/IL-17 axis. This review describes the biology and the origins of the group 3 ILCs (ILC3s) in humans, focusing on their role in the pathogenesis of axSpA.

Recent Findings Clinical trials showed the effectiveness of IL23/IL-17 axis inhibition in both spondyloarthritis (SpA) and Inflammatory Bowel Disease (IBD). Recent findings confirm the high prevalence of subclinical gut inflammation in patients with SpA. Translational data in humans have demonstrated an increase in the number of ILC3s responsive to IL-23 and producing either IL-22 or IL-17 in the gut of SpA patients. The observation of gut-derived ILC3s in circulation and at inflamed tissues in patients with SpA suggest a recirculation of ILCs from mucosal site to lymphoid tissues and possibly enthesis and joints.

Summary Multiple observations demonstrate the expansion of IL-17- and IL-22-producing ILC3 in the subclinically inflamed gut of SpA patients. These innate immune cells, also observed in normal entheses, seem to be able to re-circulate from the gut to inflamed tissues of SpA patients, thus contributing to the disease perpetuation. The development of tools that can provide access to diseased tissue from sacroiliac joint and spinal entheses will provide valuable knowledge on the role of ILC3 in axSpA pathogenesis.

Keywords Group 3 innate lymphoid cells · Lymphoid tissue inducer cells · Gut inflammation · Spondyloarthritis · Ankylosing spondylitis · IL-17 · IL-23/IL-17 axis

Introduction

The knowledge on the relevance of the interleukin-23-interleukin-17 (IL-23-IL-17) pathway in spondyloarthritis (SpA) pathogenesis has strongly contributed in markedly improving the therapeutic options.

✉ Francesco Ciccia
   francesco.ciccia@unicampania.it

1   Dipartimento di Medicina di Precisione, Section of Rheumatology, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy

2   Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), Università degli Studi di Palermo, Palermo, Italy

3   Dipartimento di Biopatologia e Biotecnologie Mediche, Università di Palermo, Palermo, Italy

In the last few years, it has become increasingly clear that the main source of IL-17 in patients with SpA is represented by innate immune cells, such as γδ T cell, mucosal-associated invariant T (MAIT) cells, neutrophils, and innate lymphoid cells 3 (ILC3s) [1, 2, 3••, 4, 5••]. Innate lymphoid cells (ILCs) belong to a heterogeneous family of immune effector cells, which have recently gained a great interest in immunology and rheumatology. ILCs are mainly tissue-resident cells, especially situated at epithelial barrier surfaces, where they are involved in early and prompt immune response against pathogens, in tissue remodeling and in maintaining of organ homeostasis [6, 7]. Recent evidence demonstrated that ILCs dysregulation is implicated in the pathogenesis of several inflammatory and immune-mediated diseases [8].

In the intestine, strict cross-talk between microbiota, tissue-resident macrophages and ILC3 producing IL17 and IL-22 seems to be essential for mucosa homoeostasis, and perturbation of this interaction can lead to local and systemic inflammation. Beyond the intestine, ILC3s have been demonstrated

to be present in normal human entheses and to be expanded in the peripheral blood and inflamed tissue of patients with ankylosing spondylitis (AS) and those with psoriatic arthritis (PsA). Most of the mechanistic data now available on ILC3 development and biology come however from animal models. This review provides an overview of the most relevant human evidence linking ILC3 with AS and PsA pathogenesis and also highlights the still unanswered questions.

Classification of ILCs and Ontogeny

The earliest identified ILCs belonged to a single group of cytotoxic cells, conventionally named natural killer cells (NK), which are similar and complementary to $CD8^+$ cytotoxic cells, and lymphoid tissue inducer (LTi) cells, that are crucial for the development of lymph nodes during embryogenesis [9, 10]. Works in mice and humans led to the identification of further subtypes by transcription factor expression patterns, cytokine response, and cytokine production.

Based on the most recent data, ILC are further classified into three main groups: (1) conventional NK (cNK), intraepithelial ILC1 (ieILC1), and ILC1; (2) ILC2 group; and (3) ILC3 and LTi cells. However, recent evidence suggests a certain degree of plasticity rather than a terminal differentiation, in line with the ability of these cells to adapt to different micro-environments [11•, 12, 13]. The variegated phenotype, the complex network of transcription factors, and their plasticity make the classification still challenging. Most of the data available today come from mice and are only partially replicated in human. Overall, the classification and the ontogeny of the ILCs remain a developing field and, therefore, caution should be taken in the translation of murine data to humans. However, in both human and mice, the presence and dependence of ILC on the cytokine receptor γc is clear. The γc receptor is a shared component of multiple cytokine receptors including IL-15, IL-2, IL-9, and IL-7. In particular, IL-7 receptor is essential for the development of most of the ILCs as the absence of IL7Rα is associated with altered differentiation of ILCs in both human and mice [14, 15, 16].

In human, NK precursor cells have been found in human fetal liver, bone marrow (BM), cord blood, and adult tonsil [17]. Scoville et al. identified, in secondary lymphoid tissue, a $CD34^+ROR\gamma T^+CD45RA^+$ innate lymphoid tissue precursor (ILCP) with the potential to give rise to all the ILC groups [18]. Similarly, a hematopoietic progenitor cell (HPC) characterized by CD34 positivity and sharing common features with ILC3s has been identified in human tonsil and intestinal lamina propria (LP) [19••]. According to Montaldo et al., these $CD34^+ROR\gamma T^-$ HPCs can potentially still differentiate into both NK and ILC3s whereas $CD34^+ROR\gamma T^+$ HPC are lineage-specified progenitor of ILC3s [19••] and the fate of HPCs seems to be influenced by the aryl hydrocarbon receptor (AhR) and IL-15 [19••]. Only in 2017, circulating human ILCPs $Lin^-CD127^+CD117^+IL1R1^+$ were discovered in lymphoid and non-lymphoid tissues and were able to differentiate into all ILC subsets. This observation strongly supports the in situ differentiation in response to local signals (pathogens, inflammation, and autoimmunity) [20••].

The transcription factors (TF), involved in the differentiation have been investigated mainly in the mice. The TFs inhibitor of DNA binding 2 (id2), GATA binding protein 3 (Gata3), and nuclear factor IL-3 inducer (Nfil3) have been reported as crucial during the ILC progenitor differentiation in mice [14].

Specifically, Id2 represses the activity of E-box transcription factors (E2A, E2-2, HEB), essential in the early stages of T and B cell development, whereas Gata3 suppresses B cell differentiation, through the block of EBF1 [21–27]. Both NK and LTi cells require the thymocyte selection-associated HMG box factor (TOX) and Id2 for their differentiation. T-box transcription factor (T-bet) and Eomesodermin (Eomes) characterize the NK cell while LTi cells need the transcription factor RAR-related orphan receptor γt (RORγt) [28–32].

Each population of ILC is characterized by specific transcription factors and associated cytokine expression profile. ILC1, ILC2, and ILC3 reflect the functional polarization and cytokine expression of T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells, respectively, resembling the counterpart of T cells in the innate immune system [31, 33]. Thus, the current classification of ILCs is based on their functional profile and mirrors Th cell nomenclature [34].

Group 1 ILCs

The group 1 ILCs were initially thought to encompass the interferon gamma (IFN-γ)-producing innate cells: conventional NK cells and ILC1s cells [29]. The TF T-Bet is present in the whole group; in addition, the positivity for the TF EOMES and CD103 plus lack of CD127 distinguishes the NK and intraepithelial ILC1 (ieILC1) from other ILC1s [16, 35••]. Group 1 ILCs are mainly tissue-resident cells responsive to interleukin 15 (IL-15), IL-12, and IL-18 and are involved in the primary defense against intracellular pathogens and cancer immuno-surveillance. Similar to Th1 cells, ILC1s promptly secrete tumor necrosis factor alpha (TNFα) and interferon gamma (IFN-γ), but lack significant cytotoxic activity compared to conventional NK (cNK) cells [36]. The ieILC1s have been only recently identified in gut epithelium and tonsils. These cells phenocopy the tissue-resident memory (Trm) cells [37], as are characterized by the production of TGF-beta and the expression of CD103 and CD49a [38].

Group 2 ILCs

ILC2s are tissue-resident cells, featured by the production of type 2 cytokines, such as IL-4, IL-5, IL-13, and IL-9, in response to IL-25, thymic stromal lymphopoietin (TSLP) and IL-33 [35••, 39]. Similar to Th2, ILC2s strictly depend on the high levels of the TF GATA3 [40, 41], and express the chemoattractant receptor expressed on Th2 cells (CRTH2) [42]. ILC2s were initially identified in the human fetal gut tissue and then described also in adipose tissue [43], skin [44], mesenteric lymph nodes, spleen [14], airway, lung, and in Peyer's patches [45]. ILC2s contribute to the first line of defense against helminth and nematodes [46]. In this context, ILC2s limit helminth invasion, through the production of IL-5 and IL-13, which determine the tissue eosinophils recruitment and activation, epithelial and goblet cell hyperplasia followed by increased mucus secretion. [47, 48]. ILC2s are also involved in allergic asthma, chronic sinusitis, nasal polyposis [49], atopic dermatitis [50], and Crohn's disease [13].

Group 3 ILCs

Group 3 ILCs are mainly situated at mucosal barriers, where they are involved in response to the extracellular pathogens (bacteria, fungi) and in maintaining gut homeostasis [51••]. ILC3 consists of two cell types, LTi cells (lymphoid tissue inducer cells) that are important to produce secondary lymphoid organs in embryogenesis and cells designated as NCR$^+$ ILC3 and NCR$^-$ILC3 depending on whether they express NCR receptors of NK cells capable of recognizing pathogenic molecules. [35••, 52]. In human, in fact, the expression of a 44-kD surface molecule, NK cell-associated receptor 44 (NKp44), similar to the natural-cytotoxicity-receptor (NCR) NKp46 observed in mice, distinguishes NKp44$^+$ and NKp44$^-$ ILC3s [53]. The cytokine repertoire mirrors this distinction as NKp44$^+$ ILC3s produce IL-22 and the NKp44$^-$ ILC3s mainly IL-17, representing respectively the counterpart of Th22 and Th17 [5••, 35••, 54]. ILC3s, in common with the other ILC groups, largely rely on IL-7 stimulation in addition to IL-2 as a signal for survival and proliferation. The activation is mainly controlled by the interaction in situ with the dendritic cells (DC) and CX3CR1 mononuclear phagocytes secreting IL-1 and IL-23, which lead to a prompt induction of IL-17 and IL-22 [11•, 55, 56].

The Lymphoid Tissue inducers (LTi) cells are fundamental for the formation of lymphoid structures; accordingly, LTis were described in the lymph nodes and Peyer's patches [57], being the most abundant ILCs in the ileum and colon [58]. Only recently, LTi cells have been included in group 3 ILC for the high similarity with the ILC3, including the production of IL-17 [59]. In particular, the expression of neuropilin (NRP-1) is limited to LTi and usually is absent in NKp44$^-$ ILC3s [60].

Recent evidence, suggest a role of LTi in the formation of ectopic lymphoid structures associated with chronic inflammation [60]. Studies in animal demonstrated the initial appearance of LTi type of ILC3 early in the gut during embryogenesis with the absence of ILC3 NKp46$^+$. Only after birth, they proliferate outnumbering the other ILC3 subsets [61]. On the light of this observation, a role for the environmental factor in driving the expansion of ILC3 has been demonstrated: microbiota and diet recognized as two relevant players [62]. The qualitative and quantitative alteration has suggested a bidirectional relationship between ILC3 and the gut microbiota in animal model lacking ILC3 [63].

ILC3s have a role in the compartmentalization of the microbiota as the lack of ILC3s was associated with immunization toward commensal bacteria due to a breach in the intestinal epithelial barrier [64]. On the other hand, commensal bacteria and related products foster the activation and expansion of ILC3s in the gut [61, 65]. It is worth noting that ILC3s lack pattern recognition receptors (PRR) such as Toll-like receptors that are able to sense microbial products. Therefore, an intricate network of intercellular communication and soluble mediators is in place to activate ILC3 in the presence of danger signals. In particular, dendritic cells and macrophages have been identified as a major source of IL-23, which stimulates and activates ILC3s. In this regard, a specific population of intestinal mononuclear phagocytes (MNP) CX3CR1$^+$ has been suggested as a bridge immune response from the intestinal lumen to the innate immune system [56]. More recently, it has been demonstrated that ILC3s regulate steady-state interactions between T follicular helper cells (Tfh) and B cells to limit mucosal IgA responses. ILC3s use conserved migratory cues to establish residence within the interfollicular regions of the intestinal draining lymph nodes, where they act to limit Tfh responses and B cell class switching through antigen presentation. The absence of ILC3-intrinsic antigen presentation results in increased and selective IgA coating of bacteria residing within the colonic mucosa, implicating lymph node resident, antigen-presenting ILC3s as a critical regulatory checkpoint in the generation of T cell-dependent colonic IgA and in maintaining tissue homeostasis and mutualism with the mucosal-dwelling commensal microbiota [66]. Mediators other than cytokines are involved in controlling ILC3 homeostasis and activation. Short-chain fatty acids (SCFA), in particular butyrate produced by bacterial metabolism, directly regulate ILC3s [67]. In addition, AhR ligands and vitamin A derived retinoids produced by bacterial metabolism stimulate ILCs differentiation, specifically the maturation of LTi cells and ILC3s in the adult small intestine [68, 69].

The bone marrow (BM), secondary lymphoid organs, and gut are known niches for ILC3s development, expansion, and priming; however, less is known about ILC recirculation migration programs. As stressed before, the local chemokine and cytokine milieu may be a key factor for ILC3 recruitment and

expansion in situ. Recent work demonstrated both the presence of NKp46^{+}ILC3 and ILCPs in circulation. Those cells are differently expressing α4β7, CCR7, CCR9, and CXCR6 with a known role in tissue tropism [70]. In the lung, ILC3 producing IL17A could foster the formation of ectopic lymphoid structures during chronic inflammation [60]. Similarly, the known role of IL-23/IL17 axis in inflammatory condition, such as psoriasis, IBD, and arthritis, suggests the involvement of IL-22 and IL-17 producing ILC3s in these conditions.

Ankylosing Spondylitis Associated Subclinical Gut Inflammation

The strict correlation between gut inflammation and SpA is well characterized. Its description dates back to 1976, with the analysis to the frequency of joint involvement in the course of inflammatory bowel diseases (IBD) [71]. The development of inflammatory arthritis occurs in nearly half of the patients with a diagnosis of IBD. On the other hand, up to 60% of ankylosing spondylitis (AS) patients show histologic sign of subclinical gut inflammation. Among those, only 10% will progress to a clinically and histologically defined diagnosis of IBD [72]. Analysis performed on the Gent Inflammatory arthritis, and spondylitis cohort (GIANT) revealed a correlation between the gut inflammation and the BM edema in sacroiliac joints [73•]. Recently, flow cytometry analysis showed a distinct immune signature in the colon of patients affected by AS compared to IBD in the absence of inflammation, supporting the existence of a SpA-specific gut inflammation [74•].

The gut inflammation in SpA has been classified into two main histologic presentations: acute and chronic type of inflammation. The acute type is featured by bacterial enterocolitis and increased expansion of mononuclear cells in the lamina propria [75]. The chronic type of inflammation is characterized by crypts distortion, villous atrophy, and mononuclear cell infiltration of the lamina propria lymphoid follicles. Consensual hyperplasia of goblet cells, increased mucin production, and Paneth cells activation have also been observed [76, 77].

Recent works link the subclinical gut inflammation to a certain degree dysbiosis, defined as a qualitative and quantitative perturbation in gut microbiota. Metagenomic studies involving the sequencing of the 16S ribosomal RNA gene isolated from stool samples and culture of intestinal biopsies demonstrated a significant expansion of some bacterial families, such as Raminococcus, Dialister, Prevotella, and Porphyromonas, and a relative reduction in Bacteroides species [78, 79, 80].

Our group previously demonstrated that the local inflammation and the dysbiosis are associated with the damage in the gut-vascular and gut epithelial barrier [81]. Translocation of bacterial products was observed in the submucosa and circulating blood, confirming the presence of a so-called *leaky gut* [81]. The altered intestinal permeability has been linked to the derangement of the epithelial tight-junction and higher levels of zonulin, a cellular junction modulator [81].

There is still discussion and need for further translational studies on the causal trigger of these alterations. Host-related factors such as genetics may influence the interaction between the microbiome and immune system at mucosal sites. Experiments in Lewis rats transgenic for HLA-B27 revealed a difference in cecal microbiota compared to the wild type [82, 83]. HLA-B27 is a major histocompatibility complex class I molecule with a role in preventing viral and bacterial antigens. HLA-B27 is the strongest risk factor for axSpA but not for IBD; however, common genetic predisposing factors other than HLA-B27 that are shared between AS and IBD have been identified [84]. On the other hand, we reported that bacteria isolated from the ileum of SpA patients could up-regulate ex vivo the production of zonulin from cultured cells, in support of a direct role of dysbiosis in the pathogenesis of gut inflammation in axSpA [85].

ILC3 Expansion and Recirculation in Ankylosing Spondylitis

Our previous works demonstrated the expansion of IL-17 and IL-22 producing ILC3s in the gut of patients affected by AS [5••]. The presence of these cells was also observed in peripheral blood, bone marrow, and synovial fluids, and it correlated with the Bath Ankylosing Spondylitis Disease Activity Index [5••]. On the light of these observations, the gut has been proposed as a possible *base camp* for ILC3 differentiation, expansion, and priming in AS [86]. Lyn[−]NKp44^{+}T-bet[+]RORγt[−]IL23R+ILC3s were particularly expanded in the gut of AS patients with acute or chronic gut inflammation only and were associated with increased mucins production and goblet cells hyperplasia [5••]. Specifically, the cryptopatches in lamina propria rich in LTi aggregates are sites of ILC3 maturation in the gut and cryptopatches-like structures have been observed in the gut of AS patients [5••, 87].

Moreover, in patients with AS, ILC3s were also found to be expanded in the peripheral blood, synovial fluid, and bone marrow (BM), and to express the homing integrin α4β7 [5••]. In the peripheral blood, ILC3s prevalently produced IL-22, and the production of IL-17 alone or IL-22/IL17 confined to a small subset of these cells. Among synovial fluid and BM mononuclear cells, ILC3 produced IL-22 exclusively. Besides, the mucosal vascular cell adhesion molecule 1 (MADCAM-1), the α4β7 ligand, was found to be significantly up-regulated in the gut and in the inflamed BM of patients with AS, suggesting a recirculation of ILC3 from gut to the inflamed AS tissues [5••]. In this context, inflammatory

tissues rich in MADCAM-1, IL-23, and IL-7 could be site of expansion and migration of ILC3.

Role of CX3CR1+ Mononuclear Phagocytes in Supporting ILC3 Expansion via IL-23

Intestinal mononuclear phagocytes (MNPs) are strategically positioned in the gut-associated lymphoid tissue (GALT) and in the subepithelial lamina propria [88]. In mice, lamina propria resident MNPs can be subdivided based on their surface expression of the integrin CD103 or CX_3 C-chemokine receptor 1 (CX_3CR1^+). These subpopulations of MNPs differ in terms of their ability to take up antigen and trigger intestinal T and B cell response. CX_3CR1^+ MNPs are thought to be conventional myeloid dendritic cells (DC) as they develop from a classical DC precursor [89], express CCR7, migrate to the mesenteric lymph nodes (MLNs) upon TLR stimulation, and can activate naïve T cells in vitro [90, 91]. CX3CR1$^+$ lamina propria cells do not derive from the classic DC precursors but are the progeny of Ly-6C^{hi} circulating monocytes [88]. CX3CR1$^+$ MNPs can be further divided into cells expressing high and intermediate levels of the chemokine receptor. CX3CR1$^+$ MNPs were shown to modulate adaptive immunity, through the promotion of Th17 cell differentiation [92] and FOXP3 maintenance on T reg cells [93]. Even if these cells were previously thought to be tissue-resident and non-migratory, recent data reported that during intestinal inflammation, CX3CR1$^+$ MNPs can up-regulate CCR7 and acquire the ability to migrate to secondary lymphoid organs [94]. At steady state, the microbiota inhibit the transport of commensal bacteria from the lumen to the MLNs. However, under conditions of dysbiosis, non-invasive bacteria were trafficked by CX3CR1$^+$ MNPs to the MLNs and induced T cell responses. In naïve SKG mouse intestine characterized by intestinal dysbiosis, CX3CR1$^+$ MNPs are significantly expanded [95•]. These findings suggest a central role for microbiota in regulating the antigen access from the intestinal lumen to the MLNs by a CX3CR1$^+$ cell-mediated pathway. In predisposed subjects, CX3CR1$^+$ MNPs can bridge immune response from the intestinal lumen to the innate immune system systemically.

Confirming this hypothesis, recent evidence reported that the intestinal replenishment of CX3CR1+ MNPs derives from Ly-6C^{hi} monocytes and, under inflammatory conditions, Ly6C^{hi} monocytes accumulate in secondary lymphoid organs in a DcR-6 dependent manner [96, 97]. DcR-6 is a scavenger receptor which acts as a negative regulator of inflammation, driving degradation of majority of the inflammatory CC chemokines. The control of CC chemokines by DcR-6 seems to have a particular impact on the traffic Ly6C^{hi} monocytes. In DcR-6-deficient mice, there is a delay in the clearance of inflammatory CC chemokines which promote, under

inflammatory conditions, an increased concentration of CCL2, and significant recruitment of Ly-6C^{hi} MNPs in secondary lymphoid organs [97]. Thus, the CX3CR1$^+$-mediated immune response seems to be affected by a balance between CCL2 and DcR-6 effects.

Similar data were observed in AS patients, where a significantly decreased expression of DcR-6 in the gut, BM, and synovial samples have been reported [95•]. Moreover, DcR-6 deficiency correlated with an increase in tissue and systemic expression of CCL2 as well as an increase in CX3CR1$^+$ CD59$^+$ cells (CD59 is the human homolog of Ly6). Pro-inflammatory CX3CR1$^+$ CD59$^+$, but not resident CX3CR1+ CD59− cells were expanded in the gut of AS patients compared to controls and correlated with the presence of bacteria. Transcriptome analysis revealed that CX3CR1$^+$ CD59$^+$ cells showed a specific pro-inflammatory signature in AS patients. Inflammatory CX3CR1$^+$ MNPs were also expanded in the peripheral blood and inflamed tissues of AS patients [95•]. The majority of these cells expressed the marker of intestinal homing CCR9 [98] and produce high levels of IL-23 and TNF-like molecule 1 A (TL1A) [95•]. Interestingly, the frequency of circulating CX3CR1$^+$CD59$^+$CCR9$^+$IL-23$^+$TL1A$^+$ monocytes significantly correlated with the frequency of circulating ILC3 as well as with disease activity. In the presence of isolated CX3CR1$^+$ CD59$^+$ MNPs from the gut and peripheral blood of AS patients, significant activation of ILC3 producing IL-22 was observed, indicating an important role of these cells in supporting ILC3 activation and expansion [95•]. These data suggest that in AS patients, CX3CR1$^+$CD59$^+$ monocytes may acquire a pro-inflammatory phenotype, migrating from the gut to inflamed tissues and actively participating in the activation and expansion of ILC3.

ILC3 in Enthesis

The correlation demonstrated between the ILC3 and the disease activity score in AS, in addition to the strong role of IL-23, IL-22, and IL17A in the pathogenesis of AS, point to ILC3 as a possible culprit in the AS pathogenesis and disease progression. Recently, a subset of T cells highly responsive to IL-23 has been observed in the entheses of a murine model of AS [99]. Interestingly, these murine enthesial T cells shared several immunological similarities with ILC3. These cells are Lyn$^-$, express the IL-23R and release IL-17 and IL-22, and important in driving inflammation [99]. However, the presence of ILC3 in human spinal entheses has been demonstrated only recently. McGonagle's group examined the interspinous ligament and spinous process bone of healthy individuals undergoing spinal surgery [100•]. ILC3 cells were detected by flow cytometry and expression of *RORC* by qRT-PCR. The study revealed a higher percentage of NKp44$^+$ ILC3 in enthesiaI soft tissue and peri-enthesial bone. Accordingly,

increased expression of IL-23 receptor was observed in these cells, supporting the role of IL-23 also at this site [100•].

ILC3 in Other Spondyloarthritides

Recent study results have analyzed the ILC3 role in psoriatic arthritis (PsA) [101, 102]. In particular, Soare et al. recently demonstrated that PsA is characterized by alterations of circulating ILCs. In clinically active PsA, ILC3s were significantly increased at the expense of ILC2s and the ILC2/3 ratio correlated with both composite clinical disease activity scores, but

also with imaging signs of inflammation and structural damage, supporting the concept of IL-23/IL-17 pathway activation as the driving force of inflammation in PsA patients [101]. Leijten and co-workers also demonstrated the expansion of ILC3 in the synovial fluids of PsA patients but not in peripheral blood [102]. In particular, fewer CCR6+ ILCs were found in PsA peripheral blood (PB) than in healthy control PB, while PsA synovial fluid was enriched for CCR6+ ILCs compared to PsA PB and RA synovial fluid. Increased numbers of IL-17A-producing ILC3 were present in PsA synovial fluids compared to RA synovial fluids. CCR6, NKp44, and melanoma cell adhesion molecule (MCAM) were expressed on the cell
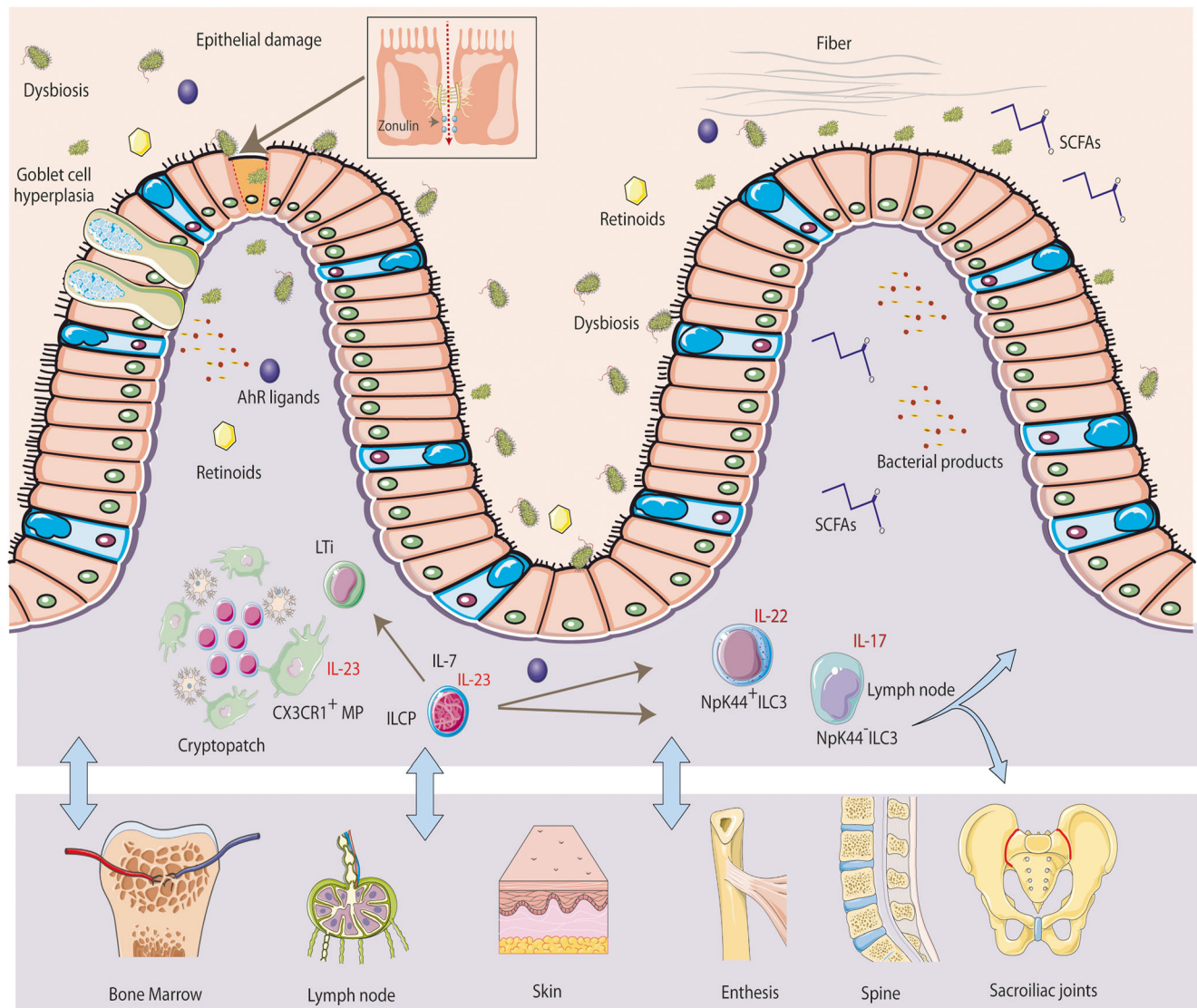

Fig. 1 Subclinical gut inflammation observed in axial-SpA is associated to dysbiosis, goblet cell hyperplasia, and altered permeability intestinal epithelial barrier. Deranged bacteria compartmentalization and increased invasiveness lead to bacterial translocation in the submucosa. Bacterial products, AhR ligands, and vitamin A derived retinoids produced by bacterial metabolism stimulate ILCs differentiation while short-chain fatty acids (SCFA) inhibit ILC3expansion. ILC3 undergo maturation and priming in cryptopatches rich in LTi within the lamina propria and supported by CX3CR1+CD59+ intestinal mononuclear phagocytes. In response to IL-23, the expanded ILC3 produce IL17 and IL-22 contributing to gut inflammation. Recirculation of ILC3 between bone marrow, secondary lymphoid organs, skin, synovium, and enthesis could participate in the articular manifestations of SpA

surface of synovial fluid ILC3s and the number of circulating NKp44^{+}, CCR6^{+}, and MCAM[+] ILCs in blood was inversely correlated with PsA disease activity.

Conclusions

There is solid evidence suggesting a pathogenic role of innate lymphoid cells and specifically ILC3s in axSpA as summarized in Fig. 1. In particular, ILC3 are significant producers of the inflammatory cytokine IL-17 in response to IL-23. The inhibition of the IL-23/IL-17 axis has been groundbreaking in the treatment of SpA. However, the available data fail to identify causes and consequences. The intestinal dysbiosis observed in the course of AS, could trigger the activation and expansion of ILC3s and possibly trigger the articular involvement. Germ-free SKG mice are resistant to the development of SpA manifestations [103, 104]. Moreover, dysregulated ILC3s can per se lead to qualitative and quantitative alteration of the commensal flora. Conversely, recent studies in mice have demonstrated that IL-23 itself could favor the outgrowth of SpA-associated pathobionts (potentially pathological organisms which, under normal circumstances, live as a symbionts) and suppress host support for homeostatic microbiota [105]. Similarly, in humans, the TNF-blockade could lead to a reduction in the dysbiotic bacteria and candidate arthritogenic bacterial peptides [106]. Further studies are needed to elucidate mechanistically the causes of ILC3s expansion in axSpA and the impact of therapeutic manipulation of the intestinal microbiota in this disease. Multiple clues suggest the recirculation of ILC3s from the gut to the entheses; however, translational studies investigating human spinal entheses and sacroiliac joints are warranted.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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