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IL-17A-Producing Neutrophil-Regulatory Tn Lymphocytes

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Abstract

The proinflammatory cytokine IL-17A, mainly produced by specialized T cells, plays an important homeostatic role in regulating neutrophil production and blood neutrophil counts. This review will assemble and discuss the evidence for this function of IL-17A-producing cells, which are collectively called neutrophil-regulatory T cells or Tn cells. IL-17A-producing lymphocytes are most abundant in the mesenteric lymph node, where they account for 0.15% of all lymphocytes. About 60% of the Tn cells are γδ T cells, about 25% NKT-like cells, and less than 15% are CD4 T cells. These latter cells are also known as T-17 or Th_{IL-17} cells, a subset of Tn cells that also plays an important role in autoimmune diseases. IL-17A produced by Tn cells regulates the production of G-CSF, which in turn promotes the proliferation of promyelocytes and maturation of neutrophils. This homeostatic mechanism plays an important role in normal physiology and in host defense against bacterial infections. This review is aimed at highlighting the important role of IL-17A-producing T cells at the interface between the adaptive and innate immune system.

Key Words

IL-17
Neutrophil
IL-23
Homeostasis
Tn cells
G-CSF

Introduction

Blood neutrophil levels are tightly controlled. Under normal homeostatic conditions, bone marrow neutrophil production (granulopoiesis) closely matches neutrophil elimination, thus resulting in stable numbers of circulating neutrophils *(1)*. Although a homeostatic regulatory mechanism has been suspected to exist for over 15 yr *(2,3)*, the cytokine cascade regulating neutrophil production was only discovered recently *(1)*. IL-23, a heterodimeric

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cytokine sharing one subunit with IL-12, is produced in response to environmental stimuli by macrophages and dendritic cells (DCs) in gut-associated lymphatic tissue and stimulates IL-17A production by a small subset of T cells (0.2% of all splenocytes in normal C57BL/6 mice), which in turn leads to elevated G-CSF production and enhanced granulopoiesis *(4,5)*. When enough neutrophils reach their various target tissues and become apoptotic, they are phagocytosed by macrophages and DCs, leading to a downregulation of IL-23 production by these cells *(1)*. Thus, a closed-loop feedback is established that regulates neutrophil production, similar to the way erythropoietin levels in response to hypoxia regulate erythrocyte homeostasis *(2,6)*. This review is focused on the IL-17A-producing T (Tn) lymphocytes that play an essential role in neutrophil homeostasis and altered neutrophil production in disease.

IL-17A and Its Receptor

IL-17A is a member of the IL-17 family of cytokines that currently consists of IL-17A, B, C, D, E, and F, *(7–12)*. IL-17A, also known as cytotoxic T-lymphocyte-associated antigen 8, is a homodimeric glycoprotein of 155 amino acids (aa) (Fig. 1A) *(7,13)*. Human IL-17F is closely related to IL-17A with 61% aa identity. Detailed structural analysis of IL-17F has revealed that it is a member of the cysteine knot superfamily sharing similarities with nerve growth factor (Fig. 1B) *(14)*. IL-17A is secreted by activated T cells and is highly conserved between species with a 61% aa identity between human and mouse. IL-17A is detectable in blood serum, synovial fluid, and tissue culture supernatants by commercial ELISA assays *(1,13,15,16)*.

The receptor for IL-17A is interleukin 17 receptor (IL-17R). Human IL-17R is an 866 aa type I transmembrane glycoprotein (Fig. 1C) *(17,18)*. IL-17R is highly conserved between species with a 72% aa identity between human and mouse *(17)*. Currently, all secreted IL-17A is believed to be biologically active at its receptor, to which it binds with relatively low affinity K_D 72 nM *(13,14)*. Five additional receptors, IL17RB–E, have also been described which are ubiquitously expressed *(19)*. A recent study by Kramer et al. *(20)* elegantly demonstrated that IL-17R exists in a preassembled multimerized form on the plasma membrane and the binding of its ligand (either IL-17A or IL-17F) induced a conformational change in the receptor as determined by a reduction in fluorescence resonance energy transfer (FRET) levels. Interestingly, the aa sequence of IL-17R family does not exhibit homology with any other cytokine receptors in either the extracellular or intracellular signaling motifs *(17)*. IL-17R signaling varies according to cell type. In immortalized human bronchial epithelial cells (16HBE) IL-17 signals through P38 and extracellular signal regulation kinase (ERK) mitogen activated protein (MAP) kinase pathways to induce IL-6 and IL-8 release, but not through protein kinase C (PKC) or phosphoinositide 3 (PI3) kinase *(21)*. In fibroblast-like synoviocytes isolated from the diseased synovium of rheumatoid arthritis patients, IL-17 also activates the MAP kinase pathway through P38 and ERK phosphorylation as well as activating the mobilization of the transcription factor nuclear factor kappa B (NFκB) *(22)*. Upstream of the MAP kinases, IL-17R has been demonstrated to signal via the signal transducer TNF receptor associated factor 6 (TRAF6; through which IL-1β signals) but not TRAF2 (through which TNF-α signals). Indeed, it has been speculated that the intracellular signaling of IL-17R is similar to that of the Toll-like /interleukin-1 receptor pathways. TRAF6 knockout mice have been generated, however they do not survive into adulthood and there are no reports on circulating neutrophil numbers in these mice, although immature B cell numbers were reduced *(23,24)* Both NFκB and activator protein-1 (AP-1) have binding

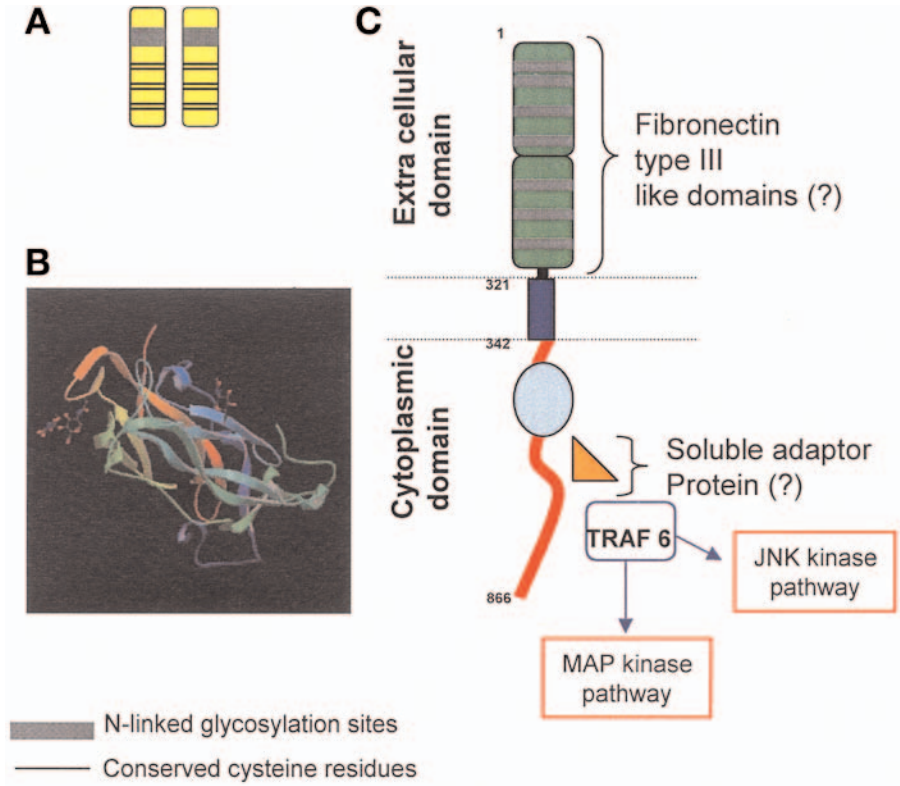

Fig. 1. A schematic diagram of human IL-17A and its cognate receptor: IL-17A is a homodimer with **(A)** one N-linked glycosylation site and six conserved cysteine residues. As yet, only the crystal structure for IL-17F has been described with the ribbon trace **(B)** presented here. The receptor for hIL-17A and F **(C)** is a type I transmembrane glycoprotein, with an extracellular domain containing seven N-linked glycosylation sites with potential structural similarity to a tandem fibronectin 3 like domain. IL-17R also has a carboxy proximal transmembrane domain and a long cytoplasmic tail *(81)*. Signaling via the JNK kinase and MAP kinase pathways occurs via TRAF6.

sites within the promoter regions of IL-17A and many other proinflammatory genes. IL-17A regulates a variety of inflammatory cytokines (i.e., IL-6), chemokines (i.e., CCL2, CXCL1, CXCL6, and CXCL8), colony stimulating factors (i.e., G-CSF and GM-CSF), and matrix metalloproteinases (MMP's) *(13,19,25)*.

IL-17A Producing Cells in Normal Mice and Humans

Several groups have recently reported that IL-17A is produced by CD4 T cells following stimulation by the newly discovered cytokine IL-23 *(1,25–28)*. This discovery is based on intracellular staining for IL-17A after blocking exocytosis of proteins by brefeldin A or similar drugs. IL-17A-producing CD4 T cells have been called Th17 *(25,28)*, Th_{IL-17} *(27,29)*, or Th1β (Mark Kaplan, unpublished) to emphasize their role as helper T cells (Th) with a unique function distinct from Th1 and Th2. Indeed, gene expression analysis of unfractionated splenocytes showed that splenocytes stimulated with IL-23, the cognate inhancer of IL-17A producing T cells, have a distinct gene expression profile quite unlike splenocytes stimulated with the Th1 cytokine IL-12 *(27)*.

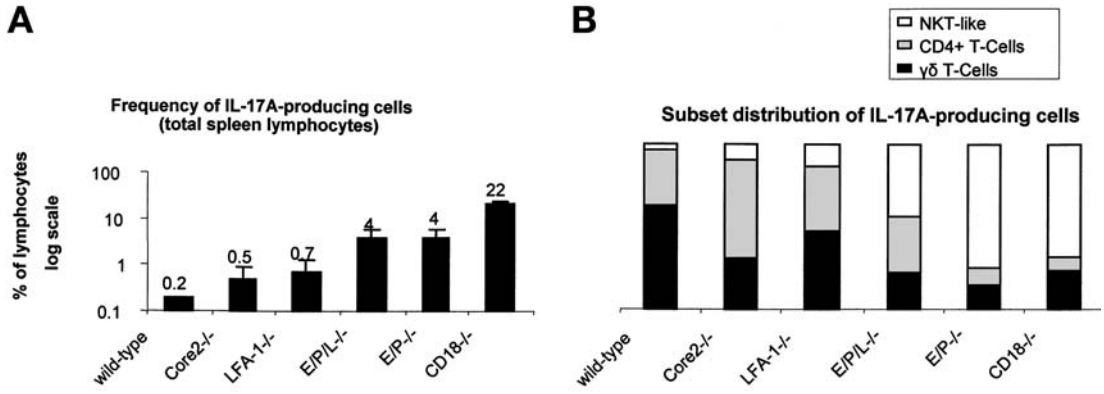

Fig. 2. Frequency of Tn cells in the spleen of wild-type and adhesion molecule–deficient mice. The percentage of IL-17-producing Tn cells as percentage of an splenocyte varies between adhesion molecule–deficient models **(A);** however, there is a significant increase in Tn cell numbers in all models compared to the wild-type mice. Data displayed on log scale. **(B)** Fractionation of Tn cells into γδ T cells (black), NKT-like T cells (white) and αβ CD4 T cells (grey). IL-17A production varies among adhesion molecule–deficient mice and the more neutrophilic mice have a larger fraction of IL-17A producing NKT-like cells compared to wild type.

The $CD4^+$ T cell population ("Th17 cells") constitutes only a minor fraction of IL-17A-producing T cells in normal mice *(1)*. In C57BL/6 mice, most IL-17A-producing cells are found in the mesenteric lymph node *(1)*. There and in the spleen most (50–60%) IL-17A-producing cells are γδ T cells (0.2% of all splenocytes) that do not express CD4 (Fig. 2). Of the remaining IL-17A-producing T cells, αβ $CD4^+$ Th17 cells, account for about 0.1% of all splenocytes and NKT cell-like with a restricted T cell receptor repertoire (preference for Vβ8) account for 0.01%. IL-17A has also been reported to be expressed at the mRNA level in neutrophils *(30)* and secreted albeit at low levels by $CD8^+$ T cells *(7,25,31)*. Current evidence suggests that most if not all of the biologically relevant IL-17A production is restricted to T cells, as nude mice (C57BL/$b^{nu/nu}$) express no detectable IL-17A protein (Stark, Burcin, and Ley unpublished data). Furthermore, nude and SCID immunodeficent mice are neutropenic; however, thymus grafts into nude mice normalizes the circulating neutrophil numbers to the same levels as wild-type BALB/c mice *(32)*. Conversely, overexpression of IL-17A in mice causes splenomegaly, increased white blood cell counts, and a 10-fold increase in circulating neutrophil numbers with G-CSF levels transiently increasing for 24 h after adenovirus-mediated gene transfer *(33)*.

In mice lacking neutrophil adhesion molecules, chemokines, or chemokine receptors (Fig. 2B), the number of Tn cells is grossly elevated. The proportion of γδ vs αβ (NKT-like) vs $CD4^+$ αβ T cells is altered in a systematic fashion. While γδ T cells are the most abundant IL-17A producers in wild-type mice, the αβ NKT-like cells are more abundant in the severely compromised mice with very high neutrophil counts. This suggests that IL-17A production is tightly regulated, and that the NKT-like cells are recruited to produce more IL-17A under emergency conditions. Surprisingly, both the NKT-like cells and the γδ T cells have not been as thoroughly investigated as the $CD4^+$ Th17 cells, although the former account for the majority of all IL-17A-producing cells. In wild-type mice, all IL-17A-producing T

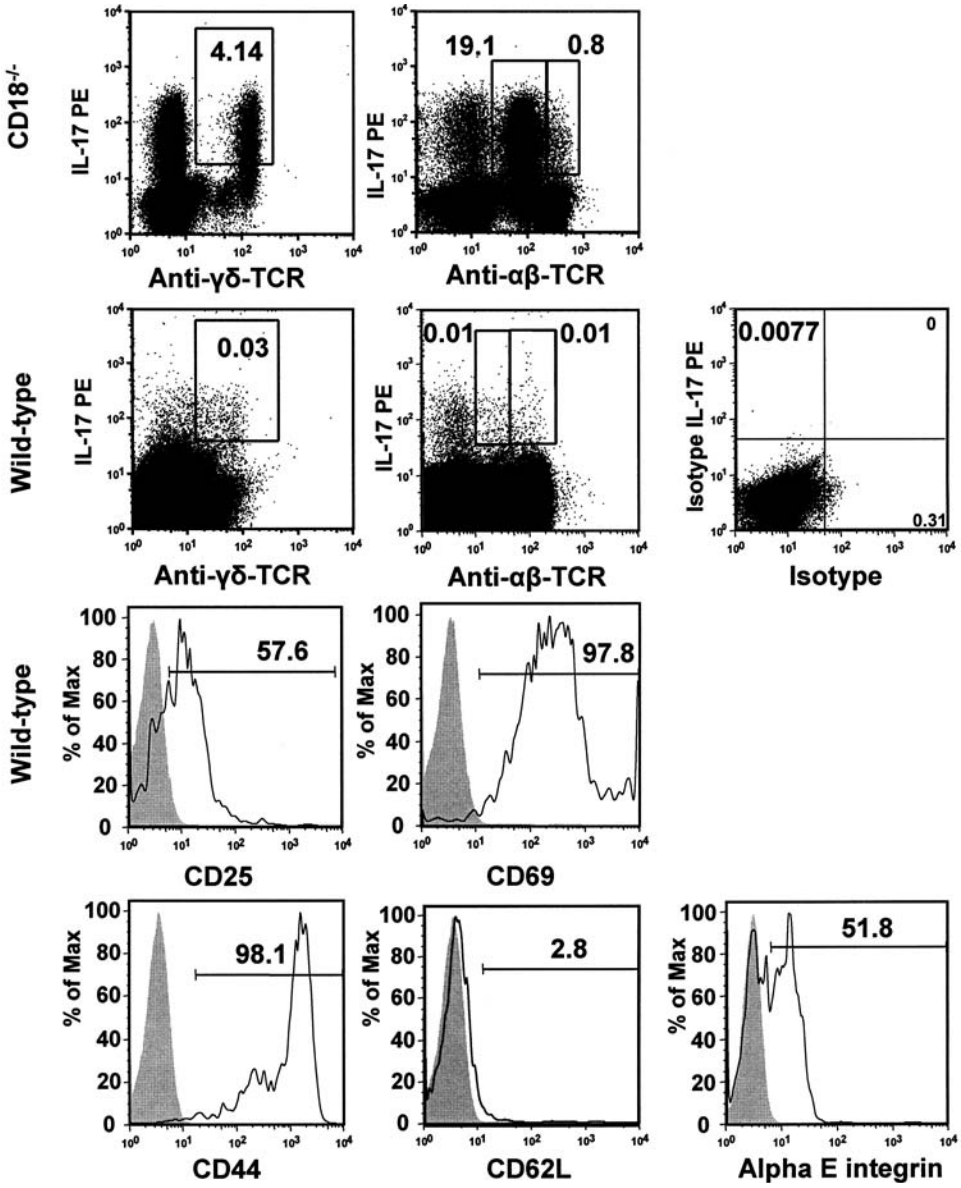

Fig. 3. Characterization of Tn cells in the spleen of healthy and severely neutrophilic mice. Splenocytes from either **(A)** CD18$^{-/-}$ or (**B** and **C**) wild-type mice were stimulated for 5 h with PMA (10 ng/mL) and ionomycin (500 ng/mL) in the presence of GolgiStop, then stained for both surface antigens and intracellular IL-17. Histogram plots **(C)** were gated on all IL-17 positive cells for analysis of activation markers represented by solid lines. The shaded area represents corresponding isotype control.

cells express CD69 and high levels of CD44, about half of them express CD25 and α_E integrin (CD103), and all are L-selectin (CD62L) negative (Fig. 3).

CD18$^{-/-}$ mice lack all four β_2 integrins and have high levels of IL-17A in the plasma (68 ± 30 pg/mL) *(5)* and develop chronic dermatitis with extensive facial and submandibular

skin erosions. Their phenotype includes elevated neutrophil counts, increased immunoglobulin levels, lymphadenopathy, splenomegaly, and abundant plasma cells in skin, lymph nodes, gut, and kidney *(34)*. One intriguing possibility is that the intensity and incidence of the inflammatory phenotype is commensurate with the high levels of IL-17A. In a transient IL-17A overexpression study, adenovirus-mediated gene transfer of the murine IL-17 cDNA targeted to the liver resulted in a transiently transgenic phenotype with a 10-fold rise in the absolute neutrophil count, a doubling of spleen size, and an increase in frequency of highly proliferative potential colonies, CFU-granulocyte- macrophage and CFU-granulocyte-erythrocyte-megakaryocyte-monocyte *(33)*. Inflammatory disease was not reported. Schwarzenberger et al. measured mIL-17 plasma levels using a bioassay based on IL-17A-stimulated IL-6 release from 3T3 fibroblasts. They expressed IL-17A levels in units, which are not directly convertible to pg/mL. However, the transient transgenics showed a 10-fold increase of plasma IL-17A above normal non-transgenic or vector control mice, suggesting that the level of IL-17A was roughly similar in the transient transgenics and $CD18^{-/-}$ mice *(5,33)*. Both observations make it clear that murine IL-17A is a cytokine that can stimulate granulopoiesis in vivo, but also has proinflammatory effects.

Even higher levels of plasma IL-17A are reached in mice lacking both CD18 and E-selectin (120 ± 30 pg/mL). These mice do not reach adulthood and have 55-fold elevated blood neutrophil counts *(35)*. Since the mice died at weaning, the inflammatory phenotype was not fully investigated, but ulcerative dermatitis and neutrophil accumulation in the lungs were observed, which may be related to the early lethality *(35)*. In mice lacking CD18 and P-selectin ($CD18^{-/-}P^{-/-}$), IL-17A levels were higher still at 215 pg/mL. These mice did not gain weight after weaning and died between 10 and 30 wk of age *(35)*. Some $CD18^{-/-}P^{-/-}$ mice developed ulcerative dermatitis and conjunctivitis. They also showed severe lung pathology with interstitial neutrophil accumulation, hemorrhage, and exudate. The lungs were also colonized with bacteria (10 of 16 analyzed), as were 10 of 16 livers and 4 of 14 spleens *(36)*. In adhesion molecule–deficient mice, it is impossible to know whether the inflammatory disease is secondary to the elevated IL-17 levels or caused by the bacterial colonization that remains unchecked because of the severe defect in neutrophil migration. Interestingly, mice lacking CD18 and IL-17R do not survive past the age of 3 mo (Stark, Bruce, and Ley, unpublished observations), suggesting that elevated IL-17A levels in $CD18^{-/-}$ mice serve an important compensatory role.

IL-23 and Its Receptor

In vitro, the combination of IL-6 and transforming growth factor β1 (TGF-β1) can induce IL-17A production in naive CD4 T cells *(37)*. IL-17A production is greatly enhanced when IL-23 binds its cognate receptor, IL-23R, on activated memory T cells *(26)*. IL-23 is a heterodimer composed of the unique IL-23p19 chain linked by a disulfide bridge to the common p40 chain (also known as IL-12p40) that it shares with IL-12 (Fig. 4A) *(38)*. Until recently, measuring p40 was thought to be sufficient to detect IL-12, and many older publications refer to IL-12 when p40 is actually measured *(39,40)*. Knockout mice lacking p40 are deficient for both IL-12 and IL-23, while p35 knockout mice lack only IL-12 and p19 knockout mice lack only IL-23 *(29,41,42)*. Human IL-23 is a 189 aa polypeptide (196 aa in mouse) similar in structure to IL-12p35 (Fig. 4B) with a 74% aa identity between human and mouse *(38)*. Interest-

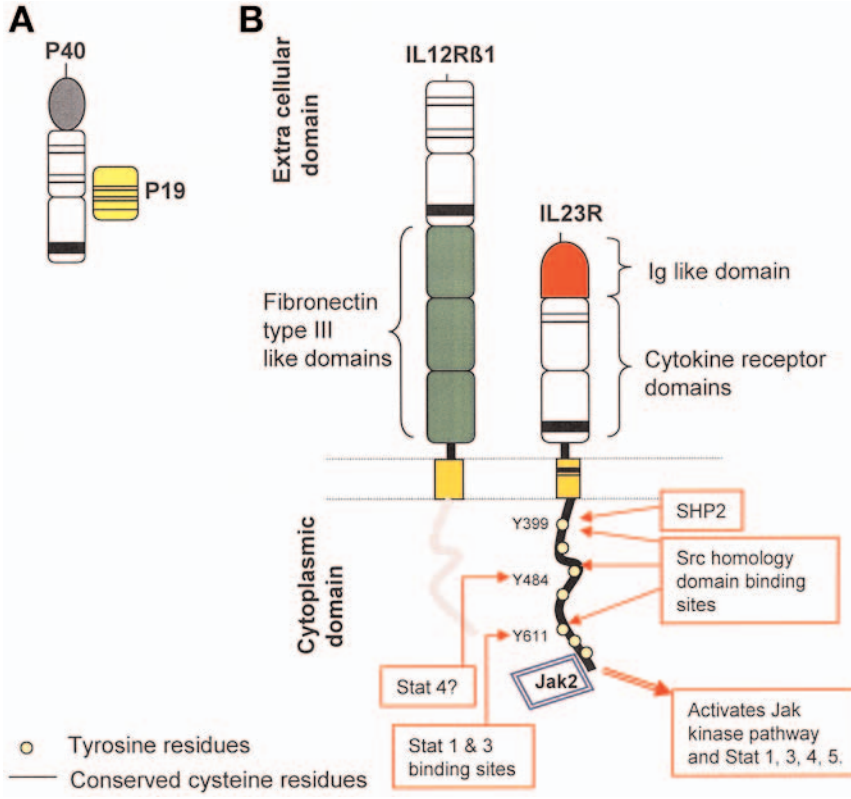

Fig. 4. A schematic diagram of the IL-23p40/p19 complex and its cognate receptor: IL-23 is a heterodimer comprised of **(A)** two subunits; the common p40 subunit that it shares with IL-12 and the unique p19 subunit. The receptor for IL-23 forms a heterodimer **(B)** comprised of the IL-12β1R and the IL-23R. Both receptor subunits consist of an extracellular N-terminal Ig-like domain, two cytokine receptor domains, a transmembrane domain, and a cytoplasmic tail. The cytoplasmic tail of hIL-23R contains seven tyrosine residues through which it signals via a number of well-defined signaling pathways including Stat 1, 3, 4, and 5, SRC homology 2, SHP2 and JNK kinase *(48)*.

ingly, purified p19 has no biological activity without forming a heterodimer with p40 and it must be co-expressed with p40 to be efficiently secreted from cells *(38)*.

The cDNA for IL-23 is expressed in various tissues and highly expressed in macrophages, DCs derived from peripheral blood monocytes (but not bone marrow–derived DCs) and polarized Th1 cells *(38)*. In DCs and macrophages, IL-23 is secreted in high concentrations upon stimulation by tumor necrosis factor-α (TNF-α), Toll-like receptor (TLR) agonists such as bacterial lipopolysaccharide (LPS) and by CD40 ligation by CD40L as well as in smaller quantities by Fas ligand *(38,43–46)*. Activated macrophages and DCs are able to secrete IL-12 and IL-23 simultaneously *(38)*. However, these cells may also be able to selectively produce either cytokine, although the putative switching mechanism is not known. Recent studies have shown a role for IL-23 in autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis mouse models *(27,42)*. Moreover, the overexpression of p19 in transgenic mice not only drastically increases neutrophil and lymphocyte

numbers but also results in multiorgan inflammation and premature death *(47)*. The phenotype of these mice is more severe than that of IL-17A overexpressing mice *(33)*.

The p19p40 heterodimer has one known receptor called IL-23 receptor (IL-23R). Initial investigations found that IL-23 could bind to IL-12Rβ1 (but not IL-12Rβ2) and activate Stat 4; however, levels of activation were low compared to IL-12 and therefore a unique receptor for IL-23 was sought *(38)*. Parham et al. were the first group to describe and characterize the unique IL-23R and its signaling capabilities from cDNA libraries *(48)*. IL-23R is highly conserved between species with a 66% aa identity between human and mouse. The cytoplasmic tail of hIL-23R contains seven tyrosine residues, three of which are Src homology 2 domain binding sites. Src homology 2 phosphatase 2 (SHP2), signal transducer and activator of transcription 1 (stat1), stat3, and stat4 also have potential binding sites within the cytoplasmic tail of IL-23R and the tyrosine kinase Janus activated kinase 2 (Jak2) is constitutively associated with the cytokine receptor (Fig. 4B) *(48)*. Thus, the binding of IL-23 to its cognate receptor complex results in the tyrosine phosphorylation of Jak2 and tyrosine kinase 2 (Tyk2) as well as stat1, stat3, stat4, and stat5, which translocate to the nucleus *(48)*. IL-23R is expressed on a small subset of T cells (Th1 and Th0), DCs, activated macrophages, and NK cells *(48)*. Current evidence suggests that IL-23 is the primary regulator of IL-17A release from memory T cells, although in vitro assays have demonstrated that these cells are capable of producing IL-17A, albeit in small quantities, upon incubation with conditioned medium from $p40^{-/-}$ DC *(31)*. Thus, IL-17A levels may be regulated by other soluble or cell-mediated factors. IL-15 has been shown to induce IL-17A production in peripheral blood and synovial fluid mononuclear cells and CD4 T cells isolated from the spleen *(49,50)*.

G-CSF, Granulopoiesis, and Regulation of Blood Neutrophil Counts

An important downstream effect of IL-17A is the production of granulocyte colony-stimulating factor (G-CSF) *(5)*. Human G-CSF is a 174 aa peptide with five conserved cysteine residues *(2)*. The mouse homolog for G-CSF is termed colony stimulating factor 3 (granulocyte) and shares a 75% aa identity with human G-CSF *(51)*. G-CSF is produced by bone marrow stromal cells of monocyte/macrophage lineage as well as vascular endothelial cells, fibroblasts, and mesothelial cells and its expression is tightly regulated. G-CSF binds a single known receptor, G-CSFR, which is expressed on all neutrophil precursor cells in the bone marrow with the mature neutrophil expressing the highest number of receptors on its surface *(2)*. Human G-CSFR is a homodimer, consisting of an extracellular domain with one NH_2-terminal Ig like domain, a cysteine-rich double-loop domain, four fibronectin type III domains, a transmembrane domain, and an intracellular domain *(52)*. Upon the binding of G-CSF to its receptor, it causes the proliferation, differentiation, and activation of granulocyte precursors *(2)*. Interestingly, serum G-CSF levels are significantly raised in adhesion receptor–deficient mice and levels positively correlate with neutrophilia *(5)*. In addition to this, antibody blockade of G-CSF reduces circulating neutrophil levels within these murine models but does not affect IL-17 levels leading to the conclusion that G-CSF is acting downstream of IL-17 *(1,5,53)*.

The Neutrostat: A Homeostatic Feedback Loop

Neutrophil production is regulated by G-CSF, which in turn is induced by IL-17A produced by neutrophil-regulatory T cells (Tn cells). Most Tn cells express γδ TCR, while a smaller group express αβ TCR with preferred

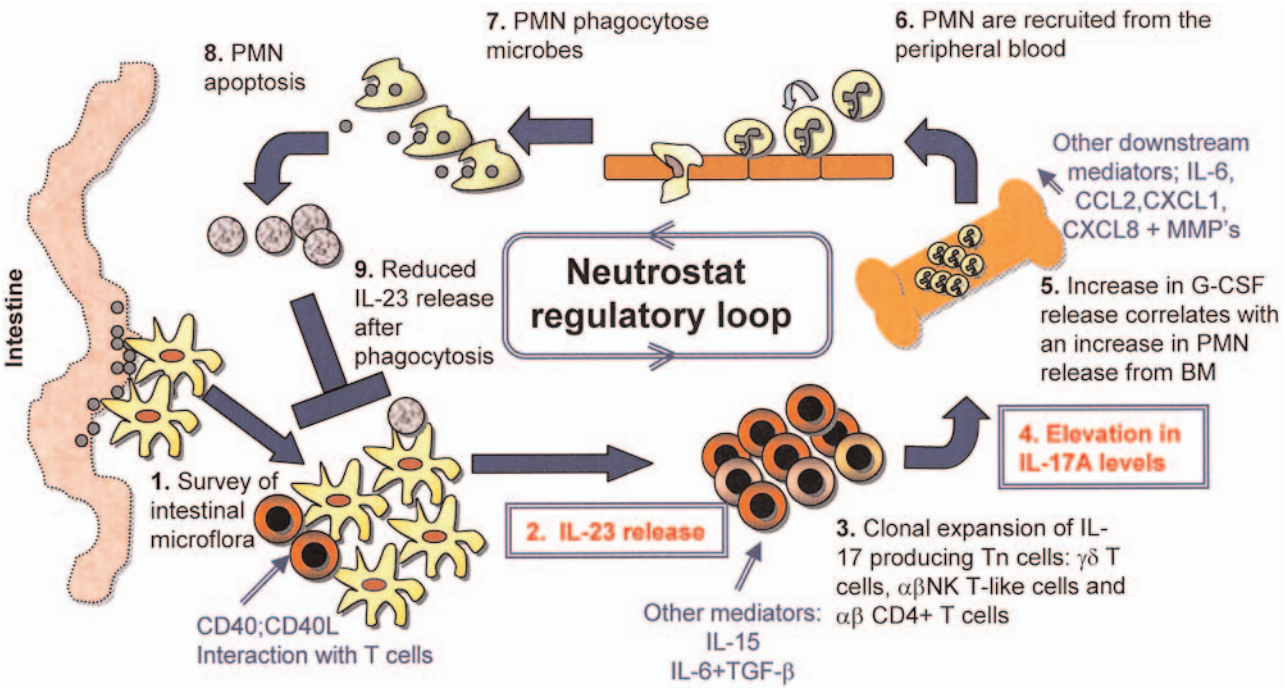

Fig. 5. The neutrostat regulatory loop. Dendritic cells (DC) situated along the intestinal tract receive signals from the gut microflora **(1).** This leads to expression of p40 and high levels of IL-23 release **(2)** by DC along specific sections of the intestine. IL-23 release may also be induced by CD40:CD40L interactions. IL-23 (and other soluble mediators, e.g., IL-15) induce the release of IL-17A **(4)** from three populations of Tn cells—γδ, NKT-like, and CD4 T cells **(3)**. IL-17A causes an increase in G-CSF (and other soluble mediators, i.e., IL-6, CXCL1, CXCL8, and MMPs) which in turn causes **(5)** an elevation in neutrophil (PMN) release from the bone marrow and **(6)** increased PMN recruitment. The circulating neutrophils are recruited into the tissue where they perform phagocytic functions **(7)** and are cleared by **(8)** apoptosis. The phagocytosis of these apoptotic neutrophils reduces IL-23 production by DCs **(9),** thus closing the feedback loop.

Table 1. Agents Regulating G-CSF Expression and Secretion

Modulators of G-CSF expression
Epidermal growth factor *(68)*
Interleukin-1 *(69–71)*
Interleukin-3 *(71,72)*
Interleukin-4 *(71,73,74)*
Interleukin-10 *(71)*
Interleukin-17A *(1,5)*
Tumor necrosis factor-α (TNF-α) *(71,75–77)*
Bacterial lipopolysaccharide (LPS) *(71,72,76)*
Granulocyte/macrophage colony stimulating factor (GM-CSF) *(78)*
Interferon-γ *(77,78)*
Phorbol 12-myristate 13-acetate (PMA) *(72,75)*

Vβ8 usage and no CD4 expression, and the remainder are classical CD4 αβ T cells. IL-17A production in these cells requires IL-23, which is provided by activated DCs and macrophages. The constitutive expression of p40 subunit in the terminal ileum and the corresponding high expression of IL-23 is probably triggered by signals generated from the local bacterial flora of the terminal ileum stimulating Toll-like receptors and other pattern recognition receptors on DCs *(4,54)*. This is supported by the observation that germ-free mice have reduced p40 expression compared to healthy mice, and germ-free rats exhibit lower blood neutrophil numbers *(4,55)* The neutrostat feedback loop (Fig. 5) is closed by neutrophils trafficking to tissues like the gut, skin, and mucosal membranes, where they become apoptotic and are taken up by resident phagocytes *(1)*. Both macrophages and DCs curb their IL-23 production after uptake of apoptotic neutrophils (or other apoptotic cells) *(1)*.

As compared to other physiologic feedback loops, the neutrostat regulatory loop is rather long, i.e., it involves many stations. This suggests that, in addition to the main pathway through IL-23, IL-17A, and G-CSF described here, other cytokines must exist that may modulate this loop. Indeed, G-CSF can be induced by a variety of cytokines (Table 1). While we know today that efficient IL-17A production requires IL-23 or IL-15, it is only a matter of time until other modulators of IL-17A are discovered *(37)*. Similarly, the full range of stimuli that induce IL-23 are not yet known. However, the phagocytosis of apoptotic cells seems to be a major mechanism reducing IL-23 expression and secretion *(1)*.

It is informative to look at the phenotype of the knockout mice lacking cytokines and cytokine receptors involved in the neutrostat pathway. G-CSF and G-CSFR knockout mice have almost the same phenotype and show an 80% reduction of blood neutrophil counts (56,57). This would be expected of knocking out a very proximal regulator of neutrophil production. By contrast, IL-17R knockout mice have only a 40% reduction in blood neutrophil counts, consistent with the more distal role of IL-17R in the loop (53). This suggests that other stimuli might compensate and induce some G-CSF. Although a IL-17-deficient mouse has been generated, the circulating neutrophil numbers were not reported (58). The IL-23p19 knockout mouse has been reported to have normal numbers of peripheral blood leukocytes, although neutrophil numbers were not specified (59). This is consistent with the notion that IL-23 is quite upstream and other cytokines may compensate for the absence of IL-23. The IL-23R knockout mouse has not been reported yet.

Knocking out genes that encode for neutrophil-trafficking molecules has effects opposite from knocking out cytokines or cytokine receptors as such interventions break the neutrostat feedback loop. Thus, interfering with the extravasation of neutrophils into tissues leads to elevated neutrophil counts (Table 2), because the neutrophils cannot reach their target tissues

Table 2. Peripheral Blood Neutrophil Counts in Wild-Type and Adhesion Molecule Deficient Murine Models[a]

Murine models	Peripheral blood neurophil counts
Wild type	†1.3 ± 0.1
$CD18^{-/-}$	†22.6 ± 1.6**
$E/P^{-/-}$	†27.0 ± 2.0**
$E/P/L^{-/-}$	7.0 ± 3.2* *(79)*
$LFA^{-/-}$	†4.6 ± 0.3**
$Core2^{-/-}$	2.4 ± 0.4 *(80)*

[a] A comparison between adhesion deficient mice and wild type mice. Data presented as neutrophil numbers x $10^3/\mu uL$ ± SEM (*$p < 0.05$, **$p < 0.0001$ by unpaired Student's t test).

† M.A. Stark, T. Burcin, and K. Ley unpublished observations. E/P, E- and P-selectin; E/P/L, triple selectin; core2, core2GlcNAc transferase.

effectively. This leads to elevated IL-23, IL-17A, and G-CSF levels, which drives elevated neutrophil production and blood counts *(1,5)*. Support for this concept comes from mixed chimeric mice reconstituted with bone marrow from CD18 knockout mice and wild-type littermates at a 1:1 ratio *(5,60)*. CD18 knockout mice have 50-fold elevated neutrophil counts *(34)*. By contrast, mixed chimeric mice, in which half of the neutrophils lack CD18 integrins, have normal neutrophil counts *(5,60)*. This shows that neutrophil production is downregulated when (some) neutrophils reach their target tissues effectively, apoptose, and become phagocytosed. Indeed, injecting normal wild-type neutrophils into CD18 knockout mice transiently corrects the neutrophilia and brings down the IL-17A and G-CSF levels *(1)*. Remarkably, in the mixed chimeric mice, the ratio of knockout and wild-type neutrophils reflects the number of transplanted bone marrow cells *(5)*. If CD18 knockout neutrophils simply accumulated in the circulation because they cannot emigrate, a higher proportion of knockout than wild-type cells would be expected.

Anti-inflammatory Effect of Phagocytosing Apoptotic Cells

Phagocytosis of apoptotic cells has strong anti-inflammatory effects on professional phagocytes some of which are mediated by TGF-β *(61–65)*. Among other cytokines, the secretion of IL-12 *(66)* and IL-23 is drastically reduced after uptake of apoptotic cells *(1)*. The mechanism by which apoptotic cells curb the inflammatory response is poorly understood. It is known that this type of phagocytosis is independent of complement and of β_2 integrins on the phagocyte *(61)*. Phosphatidylserine (PS) in the outer leaflet of the apoptotic cell is thought to play an important role, but the pathway by which PS shuts down inflammatory responses remains to be explored *(67)*.

Conclusions and Future Directions

The discovery of a homeostatic regulation mechanism that controls neutrophil production invites investigations into many of the details of its regulation. The most abundant neutrophil regulatory Tn cells are γδ T cells, followed by αβ NKT-like T cells and αβ CD4 T cells. Surprisingly, only the latter have received some attention regarding their regulation, maturation, trafficking, and precise lineage and development. The gene expression profiles of the three Tn cell subsets have not been investigated, but would be very informative. Progress in this area has been hampered by the low number of cells and the difficulty in recovering mRNA after intracellular staining. IL-23 is needed to make IL-17A, but it is not sufficient. It is unknown how phagocytes "decide" to make IL-12 or IL-23. It is likely that Tn cells play important roles in diseases, in fact, this has already been shown for the subset of Th17 cells in EAE and RA. Other downstream effects of IL-17A must be investigated.

Candidates include IL-6, CCL2, and CXCL8, but more downstream cytokines probably remain to be discovered. Tn cells are probably as important as Th1 and Th2 cells, and a rapid increase in the number of publications investigating these cells can be expected.

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