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REVIEW

Tumor Necrosis Factor and Lymphotoxin in Regulation of Intestinal Inflammation

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Abstract—Ulcerative colitis and Crohn's disease are the major forms of inflammatory bowel disease. Cytokines of the tumor necrosis factor (TNF) family play an important role in the regulation of intestinal inflammation. In this review, we discuss the function of key cytokines of this family – TNF and lymphotoxin (LT) – in mucosal healing, IgA production, and in control of innate lymphoid cells (ILCs), novel regulators of mucosal homeostasis in the gut. TNF plays a central role in the pathogenesis of inflammatory bowel diseases (IBD). LT regulates group 3 of ILCs and IL-22 production and protects the epithelium against damage by chemicals and mucosal bacterial pathogens. In addition, we discuss major mouse models employed to study the mechanism of intestinal inflammation, their advantages and limitations, as well as application of TNF blockers in the therapy for IBD.

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Key words: tumor necrosis factor, lymphotoxin, intestinal inflammation, inflammatory bowel disease, mouse models

CYTOKINES OF TNF SUPERFAMILY

Tumor Necrosis Factor (TNF), lymphotoxin (LT), and LIGHT (homologous to **l**ymphotoxin, exhibits **i**nducible expression and competes with HSV **g**lycoprotein D for binding to **h**erpesvirus entry mediator, a receptor expressed on **T-**lymphocytes, TNFSF14) are key cytokines of the TNF superfamily that perform various functions in mammalian biology. Most ligands and receptors of the TNF superfamily (over 40 ligand–receptor pairs) are expressed by immune system cells [1]. Some receptors belonging to this cytokine family, for instance, TNFR1, are able to activate mechanisms of cell death, whereas others (LTβR) predominantly promote cell survival and inflammation [2]. Most ligands of the TNF superfamily are membrane-associated trimeric molecules. However, upon activation of certain metalloproteinases, trimers may be converted into soluble form. For instance, metalloproteinase TACE (TNF-alpha converting enzyme) releases the extracellular TNF moiety [3]. Intracellular parts of receptors do not contain kinase domains and are unable to bind tyrosine kinase. Thus, signaling is realized by means of adapter molecules. Several receptors of the TNF family, such as TNFR1 and Fas, contain death domains within their cytoplasmic regions, which are able to activate and recruit caspase precursors and to induce apoptosis. Another feature of these molecules is their composition of three subunits that determines signaling stereochemistry. Thus, the listed unique structural attributes determine the connection between cytokines of the TNF superfamily and signal transduction pathways in cell proliferation, differentiation, and survival [1, 2]. This is why possible use of cytokines of the TNF superfamily and their inhibitors in therapy for various autoimmune diseases and cancer is actively studied in recent years [4].

Abbreviations: CD, Crohn's disease; DCs, dendritic cells; DSS, dextran sulfate sodium; FDA, Food and Drug Administration of USA; FDC, follicular dendritic cells; GALT, gut-associated lymphoid tissue; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; ILCs, innate lymphoid cells; LIGHT, homologous to **l**ymphotoxin, exhibits **i**nducible expression and competes with HSV **g**lycoprotein D for binding to **h**erpesvirus entry mediator, a receptor expressed on **T-**lymphocytes (another name: tumor necrosis factor superfamily member 14, TNFSF14); LT, lymphotoxin; LTβR, membrane lymphotoxin receptor; MHC, major histocompatibility complex; MNP, mononuclear phagocytes; TACE, TNF-alpha converting enzyme; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; UC, ulcerative colitis.

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The main ligand–receptor interactions in the TNF/LT system as well as producer and responding cells are presented in the Fig. 1. TNF and TNFR are widely produced. Membrane lymphotoxin is an $LT\beta_2LT\alpha_1$ heterotrimer that is predominantly located on lymphocytes. LTβR receptor for the membrane lymphotoxin, in turn, is expressed in epithelial and stromal cells, dendritic cells (DCs), and macrophages, but it is absent on lymphocytes [3, 5]. This expression pattern for LT and LTβR provides interaction of LT-producing lymphocytes – cells with stromal microenvironment – that is important for lymph node development and maintenance of lymphoid organ structure [3, 6]. Recent studies have demonstrated that $LT\alpha_1\beta_2$ has two LTβR binding sites with different affinities, and LTβR dimerizes upon $LT\alpha_1\beta_2$ binding. This dimerization is required for efficient signal transduction [7]. Soluble $LT\alpha_3$ homotrimer does not bind LTβR, but it interacts with TNFR1 and TNFR2, and, similarly to TNF, it is able to stimulate development of various inflammatory reactions. In contrast to TNFR1, which is constantly expressed in all cells, TNFR2 expression is more selective and inducible [8]. It is known that membrane TNF has higher affinity for TNFR2 compared to the soluble TNF [8]. Another TNF family ligand, LIGHT (TNFSF14), mainly expressed on T cells, macrophages, NK cells, and DCs, can also interact with LTβR [3]. In addition to LTβR, LIGHT can interact with herpes virus entry mediator (HVEM) receptor, which is also a ligand for receptors belonging to another cytokine family, CD160 and BTLA (B and T lymphocyte attenuator) [9]. This complex system of ligands and receptors in the TNF cytokine family provides selectivity of interaction and signal transduction between various immune system cells and implies involvement of compensatory mechanisms.

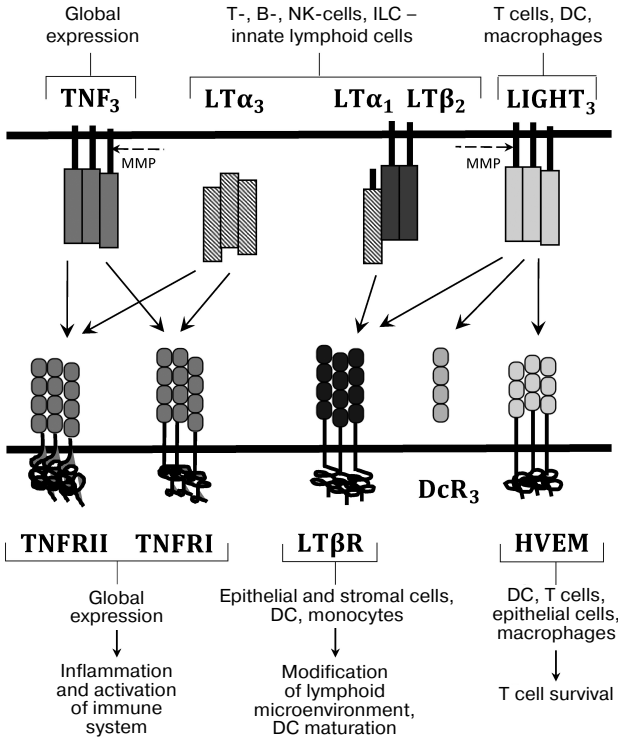

Fig. 1. Ligands and receptors of the TNF/LT family. The main ligand–receptor interactions and producer cells are indicated with arrows. The dashed line indicates action sites for metalloproteinases (MMP), whose activity converts membrane-bound ligand into a soluble form. DcR3 is a receptor-trap for LIGHT (found in some human tumors).

The best-studied function of LT and LTβR is their participation in lymphoid organ development, such as lymph nodes and Peyer's patches, and in maintenance of proper spleen white pulp architecture [6, 10]. However, it was later shown that LT function is broader and not restricted to lymphoid organs and protection against infections. Thus, it was found that LT plays an important role in maintenance of lipid homeostasis and liver regeneration. It also regulates inflammation and commensal gut microbiota, proliferation, and tumor transformation [11-17]. In addition to maintenance of lymphoid organ structure, TNF also participates in defense against infections, regulation of inflammation, and autoimmune diseases [18-20]. Therefore, LT, TNF, and LIGHT form an integral signaling network that is required for efficient innate and adaptive immune responses. We will further review mechanisms of TNF and LT participation in regulation of intestinal homeostasis and describe mouse models widely used in studying the mechanisms of intestinal inflammation.

EXPERIMENTAL ANIMAL MODELS FOR INTESTINAL INFLAMMATION

The intestine is a sophisticated organ containing different cell types that contact the "outer" environment and are constantly interacting with commensal microbiota. Revealing mechanisms involved in intestinal inflammation and damage is an important step in developing therapeutic strategies and prevention of disease. It is beyond doubt that the most reliable data can only be obtained during analysis of samples from patients who suffer from various diseases. However, sampling size, ethical issues related to utilization of human samples, and genetic heterogeneity in the group significantly restrict human research trials. Therefore, studying pathogenesis of the gastrointestinal tract requires developing complex experimental models on animals that are devoid of such drawbacks as sampling limitation and genetic heterogeneity. At the same time, human diseases should be reproduced in these animals with maximum likeness [21-23]. Advantages in the genetic engineering field and well-studied genome make mice the most-used object in modeling immune processes, including those involved in intestinal inflammation [24]. Modeling disorders in mice

gives a number of advantages, such as simplicity of genome and microbiome manipulations, and fast generation change. On the other hand, small size, different susceptibility to disease due to different genetic background, complexity of surgery, and high cost of keeping impose certain restrictions. Despite the fact that no animal model can perfectly reproduce a human disease, using mice to study mechanisms that regulate intestinal inflammation now seems optimal [21, 22, 25, 26].

Ulcerative colitis (UC) and Crohn's disease (CD) are major inflammatory bowel diseases (IBD) in humans [27-31]. The number of patients with IBD grows in developed countries. Therefore, about three million people in Europe and two million in the USA suffer from these disorders [32, 33]. Despite different etiologies, these disorders are characterized by chronic intestinal inflammation, epithelial barrier damage, and misbalance in commensal microbiota, cytokines, and immune cells, which results in development of immunopathology [27, 34-36]. Like the majority of autoimmune diseases, IBD pathogenesis depends on genetic susceptibility and ambient conditions, which may include intestinal infections, diet, stress, and disruption of the balance of microbiota [37, 38].

Many experimental mouse IBD models allow observing spontaneous development of chronic disease similar to that in humans. However, use of chemical and biological inducers of inflammation that provide fast development of reproducible reaction is also advantageous [21, 24, 39]. The diversity of chemical compounds and pathogenic microorganisms applied for induction of intestinal inflammation allows dissection of both acute and chronic inflammation [21, 39, 40]. One model is often insufficient for precise disease modeling. In this case, one has to utilize several mouse models of disease to see the whole picture. Thus, finding the most appropriate model for studying different aspects of intestinal inflammation is a key step in designing a research program. The most frequently used intestinal inflammation models along with their advantages and drawbacks are presented in Table 1.

In experiments on animals, chemical inducers are used as fast, inexpensive, and efficient strategies for intestinal inflammation modeling. DSS (dextran sulfate sodium) and TNBS (2,4,6-trinitrobenzenesulfonic acid) are frequently used for inducing colitis in mice. Acetic acid and oxazolone are applied much less often. Efficiency of intestinal tissue damage induction depends on DSS molecular mass, concentration, manufacturer, and reagent batch [39, 42]. Moreover, damage severity varies depending on genetic background of mouse line, sex, age, and microbiota composition [43, 68, 69]. One must consider microbiota composition when comparing experimental animals with a control group since differences in this parameter may cause variations in mouse susceptibility to DSS-induced colitis. Hence, using littermate controls in the same cage is recommended. If this is impossible, microbiota composition is balanced before the experiment by means of bedding exchange during a week, as mice are prone to coprophagy. Despite the difficulties arising during work with chemically induced colitis models, such agents as DSS and TNBS are valuable tools for studying pathogenesis of the intestinal inflammation [21, 23].

As an alternative to chemical agents, biological inducers of inflammation such as bacteria (*Escherichia coli*, *Citrobacter rodentium*, etc.), viruses, and helminths are applied. Change in microbiota composition may result in inflammatory response. This is why use of biological inducers of inflammation is considered to be closer to natural physiological conditions.

Most genetically engineered mouse models are designed exclusively for research applications: finding functions of molecules via complete or conditional gene knockout, stimulation, or repression of intracellular signaling pathways, withdrawal of transcription factors, and immunocompetent molecules, cells, and whole organs [24]. Immunodeficient mice were also used in studying mechanisms of response to intestinal injury. Such animal models include immunodeficient SCID and *Rag1*$^{-/-}$ mice. These mice are subject to adoptive transfer of $CD4^{+}CD45RB^{high}$ naïve T cells, which are activated by interaction with food antigens and commensal microorganisms and cause chronic transmural inflammation of the large and small intestine [57, 70]. Mouse models with spontaneous development of intestinal inflammation, such as IL-10$^{-/-}$ and SAMP1/Yit mice, most precisely reproduce Crohn's disease, a chronic intestinal disease [52, 53, 71]. However, these models also have a number of drawbacks (Table 1). "Humanized" animals with introduced human genes maximally approach a model system to human disorder [72]. For instance, rats in which human HLA-B27 allele (associated with intestinal inflammation) is expressed are successfully used as a system for testing therapeutic agents [66]. Thus, genetic engineering in animals provides a universal platform for studying intestinal inflammation mechanisms.

INNATE LYMPHOID CELLS – NEW REGULATORS OF INTESTINAL INFLAMMATION

Equilibrium between efficient immune response and pathological intestinal inflammation requires fine regulation of interactions between the intestinal epithelium and immune cells. Upon pathogen entry to intestinal mucosa, innate immune system cells act as the first line of defense. Innate lymphoid cells (ILCs) are a recently discovered family of the innate immune cells that play an important role in immune response and mucosal inflammation [73, 74]. ILCs are found in barrier tissues, where they participate in protection against pathogenic microorganisms

Table 1. Modeling inflammatory bowel disease in animals

Inducer	Pathogenesis and symptoms	Advantages and drawbacks	Reference
1	2	3	4
DSS (dextran sulfate sodium), 2-5% in drinking water for 5-7 days for acute model. Chronic model: 3 cycles: 2% DSS – 5 days and 14 days interval	DSS disrupts epithelial barrier that leads to penetration of commensal microbiota, induction of immune response and development of severe progressing colitis. Hyperactivation of NF-κB pathway results in production of proinflammatory cytokines (IL-1, TNF, IL-6, and IL-8) and adhesion molecules. Weight loss, diarrhea, rectal bleeding, piloerection, and anemia are observed in the acute phase. DSS is frequently used to study innate immune mechanisms in colitis pathogenesis	*Disease model*: UC *Advantages*: experiment simplicity, induction of both acute and chronic colitis *Drawbacks*: efficiency depends on many factors, including DSS molecular weight (routinely used 40 kDa DSS causes severe colitis, while 5 kDa DSS causes mild colitis), DSS dosage, uptake duration, genetic background (C3H/HeJ and Balb/c are more susceptible compared to C57Black6), sex (males are more susceptible). Composition of commensal microbiota in experimental groups of mice should be equilibrated	[39, 41-43]
TNBS, or DNBS, rectal administration	Inflammation develops as delayed-type hypersensitivity reaction to haptens with activation of Th1 cells and associated IFNγ, TNF, and IL-12. Main inflammatory mediators: leukotriene B4 and monohydroxy derivatives of fatty acids. Inflammation is accompanied by weight loss, rectal bleeding, diarrhea, and bowel wall thickening	*Disease model*: mainly CD, but can also be used for UC *Advantages*: transience of experiment, reproducible results, prolonged symptoms with proinflammatory immune cell infiltration and ulcers. Both acute and chronic stages can be studied *Drawbacks*: ethanol, which is used as a solvent for TNBS, may cause inflammation of intestinal mucosa regardless of immune response	[39, 44-46]
Oxazolone, rectal administration	Inflammation develops according to Th2-dependent type and is characterized by significant increase in production of IL-13, IL-4, and IL-5. Clinical manifestations are weight loss, watery stool with blood, bowel wall thickening, and erosion of distal colonic epithelium, swelling, and ulcers	*Disease model*: UC restricted to distal part of colon *Advantages*: simplicity of experiment *Drawbacks*: reproducibility of the model depends on the presence of NKT cells producing IL-13. Low oxazolone dosage leads to induction of mixed Th1/Th2 colitis	[39, 47]
Citrobacter rodentium	Wild type mice (C57Black/6) develop mild diarrhea and inflammation with hyperproliferation of epithelium. Severe inflammation with ulceration, diarrhea, and weight loss leading to death in case of deficiency in innate lymphoid cells (ILCs), or $CD4^+$ T cells and B cells, or several cytokine pathways	*Disease model*: UC and infectious colitis *Advantages*: well-characterized model for studying both ulceration and proliferative thickening of the colon *Drawbacks*: full elimination of pathogen in wild type mice takes 28 days, so this model is generally not suitable for studying chronic inflammation	[48, 49]
SAMP1/Yit mice, spontaneous ileitis	Development of inflammation is preceded by increase in epithelial permeability; further aggravation of damage is accompanied by progressive dysfunction of epithelial cells. In this model, damage is characterized by transmural inflammation and granulomatous changes in epithelium morphology. Intestinal inflammation is often accompanied by skin damage	*Disease model*: CD *Advantages*: inflammation develops spontaneously and is localized in terminal part of the small intestine that precisely corresponds to Crohn's disease symptoms *Drawbacks*: multigene disorder, mixed genetic background, various immune defects; precise cause of disease is unknown	[50-52]
IL-$10^{-/-}$, spontaneous colitis	Inflammation develops due to reaction to food antigens and commensal microbiota. Mice at the age of 2-4 months spontaneously develop transmural colitis and acute inflammation of the cecum, rectal prolapse	*Disease model:* CD *Advantages*: colitis develops spontaneously *Drawbacks*: reproducibility is about 80% depending on sex and animal housing conditions	[53, 54]

Table 1. (Contd.)

1	2	3	4
$TNF^{\Delta ARE}$ mice	Targeted deletion of AU-rich region (ARE) of TNF gene leads to increased production of TNF and development of colitis. Inflammation covers terminal part of small intestine. In addition to chronic intestinal inflammation with abundant lymphocyte infiltration, mice develop polyarthritis	*Disease model*: CD *Advantages*: symptoms and pathogenesis are the same as in CD. Spontaneous development allows studying efficiency of therapeutic TNF blockers *Drawbacks*: systemic inflammation hampering analysis of results	[55]
Adoptive transfer of $CD45RB^{high}$ $CD4^+$ cells	Inflammation is induced in recipient mice with severe mixed immunodeficiency (SCID) upon adoptive transfer of naïve $CD45RB^{high}$ $CD4^+$ T cells without regulatory T cells. Intestinal inflammation is caused by Th1/Th17-dependent inflammatory response	*Disease model*: IBD in immunodeficiency context *Advantages*: the model is a universal tool for studying immunological and genetic factors affecting development of colitis dependent on T cells *Drawbacks*: severity of developed colitis depends on donor and recipient mouse lines. This reduces reproducibility of results	[56, 57]
$IL\text{-}2^{-/-}$	Acute colitis develops because of constitutive activation of T cells and deficiency of $CD4^+CD25^+$ combined with disruption of mechanism of cell death regulation. At the age of 8-9 weeks, mice develop acute inflammation of colon and cecum, which does not involve small intestine. The disease is characterized by colonic wall thickening and ulceration	*Disease model*: UC *Advantages*: wide opportunity in studying the role of cell death in colitis pathogenesis *Drawbacks*: early lethality of the model does not allow observing chronic inflammation	[58, 59]
$A20^{-/-}$	A20 is a universally expressed ubiquitin-modifying protein that is activated by a signal through TNFR, IL-1R or NOD2 receptors. A20 represses TLR-mediated activation of NF-κB. A20-deficient mice develop spontaneous intestine inflammation, cachexia, and early death due to sustained TLR-dependent activation of NF-κB	*Disease model*: spontaneous colitis *Advantages*: spontaneous disease development *Drawbacks*: deficiency of A20, which among other things performs negative feedback regulation of TLR signaling, causing systemic damage and inflammation. This complicates data analysis	[60, 61]
$TCR\alpha^{-/-}$	$TCR\alpha^{-/-}$ knockout mice spontaneously develop chronic colitis that is mediated by Th2 cells. Clinical manifestations are watery stool, Goblet cell loss combined with infiltration by lymphocytes and neutrophils	*Disease model*: UC *Advantages*: clinical manifestations are very close to those of ulcerative colitis in humans with inflammation pattern restricted to colonic mucosa *Drawbacks*: inflammation extent depends on commensal microbiota	[62, 63]
$NEMO^{-/-}$	Inflammation is caused by pathological activation of T cell response due to lack of regulatory IKKγ subunit. Histopathological characteristics of this model include severe colitis with widened crypts, goblet cell loss, and extensive immune cell infiltration into mucosa	*Disease model*: UC and CD *Advantages*: spontaneous development of the disease *Drawbacks*: NEMO deficiency causes systemic organ dysfunction. This hampers analysis of results	[64]
IL-7 transgenic mice	IL-7 expression in colonic mucosa attracts T cells and neutrophils, whose infiltration results in chronic disease in transgenic mice	*Disease model*: UC *Advantages*: the model is useful for dissection of T-dependent colitis pathogenesis and a colitis that is characterized by mixed cell infiltrate (neutrophils and lymphocytes). It is suitable for testing therapeutics designed for suppression of T cell activation	[65]

Table 1. (Contd.)

1	2	3	4
HLA-B27 transgenic rats	Human HLA-B27 allele is associated with intestinal inflammation. HLA-B27 transgenic rats develop spontaneous intestinal inflammation that involves abdomen, small intestine, and distal part of colon. The disease is characterized by crypt hyperplasia and infiltration by proinflammatory monocytes	*Disease model*: UC and CD *Advantages*: allows studying pathogenesis of MHC-restricted diseases. This model is widely used in studying the effect of resident microorganisms on development of acute and chronic stages of inflammation of gastrointestinal tract *Drawbacks*: systemic inflammation hampers analysis of results	[66, 67]

and in damage repair. In contrast to classical lymphocytes, ILCs lack antigen-specific receptors [75-77]. Innate lymphoid cells can be divided into three groups based on variations in cytokines produced and transcription regulators involved [78-80]. The first group includes cells with the main transcription factors T-bet (ILC1) and eomesodermin (NK – natural killers); transcription factor GATA3 is important for ILC2s; innate lymphoid cells of the third group (ILC3) depend on transcription regulator RORγt [78]. Numerous studies published during the last few years have shown high polyfunctionality of ILCs. RORγt$^+$ ILC3s are involved in regulation of intestinal inflammation, antifungal and antibacterial protection, participate in control of commensal microbiota, development and repair of mucosa-associated lymphoid tissues, and carcinogenesis [73, 81-87]. Secretion of proinflammatory cytokines by activated antigen-presenting cells in the intestine activates ILC3s for IL-17a, IL-22, and GM-CSF secretion. IL-22 secretion increases production of antimicrobial peptides by epithelial cells and production of mucins, which is important for retention of commensal microbiota and for protection from bacterial and viral pathogens [84, 88, 89]. At the same time, IL-12 produced by dendritic cells inhibits the activity of ILC3s and can result in their conversion into ILC1s, which can trigger intestinal inflammation by secretion of IFNγ [90-92]. Thus, there is a differentiation plasticity between ILC populations dependent on instructive cytokines and balance between specific transcription factors [93-97]. ILC3s can also control the adaptive immune response. On one hand, ILC3s are able to boost T- and B-cell immune responses via expression of costimulatory molecules CD40L and OX40L [98, 99]. On the other hand, ILC3s induce elimination of autoreactive CD4$^+$ T cells specific for commensal microbiota antigens by expressing MHCII and presenting antigen. This suppressor phenomenon has been called intestinal T cell selection, similarly to negative selection in the thymus [85].

Recent studies have shown that ILC3s are important regulators of the intestinal inflammation. For example, epithelial damage in the DSS-induced colitis model in mice results in activation of ILC3s and production of IL-22 [17, 82]. IL-22, in turn, interacts with IL-22R on the intestinal epithelial cell and stimulates cell proliferation and production of mucins and antimicrobial peptides [88]. Moreover, it was shown that production of IL-22 protects intestinal stem cells from damage caused by donor immune cells in GVHD (graft-versus-host disease) reaction [100, 101]. However, under certain circumstances, ILC3s are also able to promote intestinal inflammation. For instance, high IL-23 levels can trigger production of IL-17 and IFNγ by ILC3s [102-104]. Thus, both secretion of certain cytokines by ILCs and specific ILC subpopulations can either inhibit or promote intestinal inflammation depending on the tissue microenvironment.

Identification of ILCs as key regulators of intestinal inflammation provides new opportunities in designing therapeutic approaches for human diseases. So, during recent studies of RORγt inhibitors, a selective blocker of RORγt was found for Th17 cells that does not affect the function of RORγt$^+$ ILC3s [105]. Moreover, it was suggested that low efficiency of a number of experimental therapeutic drugs for IBD is linked with their inability to target ILCs, important regulators of intestinal inflammation [106]. Thus, the design of specific stimulators and antagonists of ILC function for use in therapy for IBD seems to be a timely problem in this field.

LYMPHOTOXIN – A NEW REGULATOR OF ILC3s AND IL-22

Recent studies revealed LT as a key regulator of ILC3s in the intestine [16, 81, 107], while LTβR not only mediates interaction between epithelial cells and immune cells, but also stimulates migration of neutrophils into sites of infection and promotes regeneration of epithelial cells via activation of IL-22 producing ILC3s [17, 81, 107]. These mechanisms are required for repair of damaged epithelium and for protection from bacterial intestinal pathogens. Therefore, LTβ- and LTβR-deficient mice develop severe intestinal inflammation upon

C. rodentium infection and die during the first two weeks after infection [81, 108]. Use of mice bearing a specific deletion of the *Ltb* gene in RORγt expressing cells (RORγt-*Ltb*$^{-/-}$) revealed a key role of membrane LT on ILC3s for providing communication between ILC3s and mucosal cells, and production of IL-22 and antimicrobial peptides for protection against *C. rodentium* [107]. Interestingly, LT regulates IL-22 secretion by ILC3s indirectly via activation of accessory cells, mononuclear phagocytes (MNP) [109, 110]. The intestinal mononuclear phagocyte system includes functionally connected cell populations of bone marrow precursor cells (dendritic cells, DC), blood monocytes, and tissue macrophages [109, 110]. DCs and macrophages are a major source of IL-23, which is required for induction of IL-22 expression by ILC3s. Upon interaction between ILC3s expressing membrane LT complex and MNP expressing LTβR, activation of IL-23 production occurs, which triggers expression of IL-22 by ILC3s. It is suggested that this positive feedback loop between ILC3s and DC is required for providing efficient protection against a pathogen [107]. The scheme of interactions of ILC3s, MNP, and epithelial cells upon damage of intestinal epithelium as well as control mechanisms with participation of LTβR is shown in Fig. 2.

ROLE OF LT AND TNF IN IgA PRODUCTION IN INTESTINE AND IN REGULATION OF COMMENSAL MICROBIOTA

The gastrointestinal tract is densely populated by a variable bacterial community comprising about 100 trillion microbes of 500-1000 species [111]. Symbiotic relationships are established between the host organism and commensal microbiota. This facilitates development of the immune system and maintenance of normal physiology [112]. Constant interaction between intestinal microbes and the host immune system provides stable immune response that includes tolerance to food antigens and commensal microorganisms, and efficient response to pathogens [113]. In the gut, the immune system is comprised of Peyer's patches, mesenteric lymph nodes, isolated lymphoid follicles, and diffused lymphoid tissue associated with epithelium and *lamina propria* [10, 114]. Homeostasis between intestinal microbiota and host tissues is maintained by several mechanisms including secretion of IgA antibodies, which are able to block attachment of pathogens to the epithelium and neutralize toxins [115, 116]. Transformation of B cells localized in Peyer's patches or in the *lamina propria* from IgM-producing cells into IgA-producing plasma cells may occur by T cell-dependent or T cell-independent mechanisms [116-119] shown in Fig. 3.

The T cell-dependent IgA production pathway takes place mainly in Peyer's patches and depends on germinal center formation and interactions of B cells with follicular helper T cells (T_{FH}) [120]. Lymphoid patch of the cecum also participates in production of IgA [121]. T cell-independent IgA production pathway occurs predominantly in isolated lymphoid follicles (ILF) and in own proper mucous plate (*lamina propria*) [122, 123]. LTβR is required for development of gut-associated lymphoid tissue (GALT), including Peyer's patches, ILF, and mesenteric lymph nodes [10, 124].

Formation of Peyer's patches, ILF, and mesenteric lymph nodes requires expression of LT on the surface of lymphoid tissue inducer cells (LTi), which interact with LTβR expressing stromal cells during embryogenesis and trigger the development of lymph nodes and Peyer's patches [10, 125, 126]. TNF also participates in the development and maintenance of lymphoid organs in the intestine [18, 127], though to a lesser extent compared to LT. Therefore, in contrast to LTβR$^{-/-}$ mice, TNF$^{-/-}$ and TNFR$^{-/-}$ mice develop mesenteric lymph nodes, ILF, and, in some strains, Peyer's patches [10, 127]. Twenty-five percent of LTβ$^{-/-}$ mice develop mesenteric lymph nodes, which display a disturbed microstructure [10, 127]. Production of IgA in mice with a deletion of the membrane lymphotoxin $LT\beta_2LT\alpha_1$ (LTβ$^{-/-}$ mice) and in LTβR$^{-/-}$ mice is sharply decreased, in contrast to slight reduction of IgA production in TNFR1$^{-/-}$ and TNFR2$^{-/-}$ mice [16, 128].

It is worth mentioning that the defect in IgA production in the gut of LTβR$^{-/-}$ and LTβ$^{-/-}$ mice cannot be fully explained by the defects in GALT development. In fact, recent studies have shown that in mice with deficiency of $LT\beta_2LT\alpha_1$ on RORγt$^+$ ILC3, IgA levels in the intestine remains normal, despite the absence of GALT [16, 128]. Detailed experiments have demonstrated that $LT\alpha_1\beta_2$ from RORγt$^+$ ILC3 is required for the T cell-independent IgA induction in the *lamina propria* via DC activation. At the same time, soluble LT homotrimer ($LT\alpha_3$) secreted by RORγt$^+$ ILC is required for T cell-dependent induction of IgA in the *lamina propria* by promoting T cell migration to the gut [16, 128] (see also Fig. 3).

Thus, members of the TNF superfamily play a key role in formation of isolated lymphoid follicles, and IgA production in the intestinal tissues, which is required for control of commensal microbiota, maintaining its proper composition and timely elimination of pathogens and toxins neutralization.

APPLICATION OF TNF BLOCKERS IN THERAPY OF INFLAMMATORY BOWEL DISEASE

Patients with inflammatory bowel disease (IBD) suffer from chronic pains, bloody diarrhea, and weight loss. Such IBD-associated syndromes as skin inflammation and joint pain are also frequent [27, 129]. Two main forms

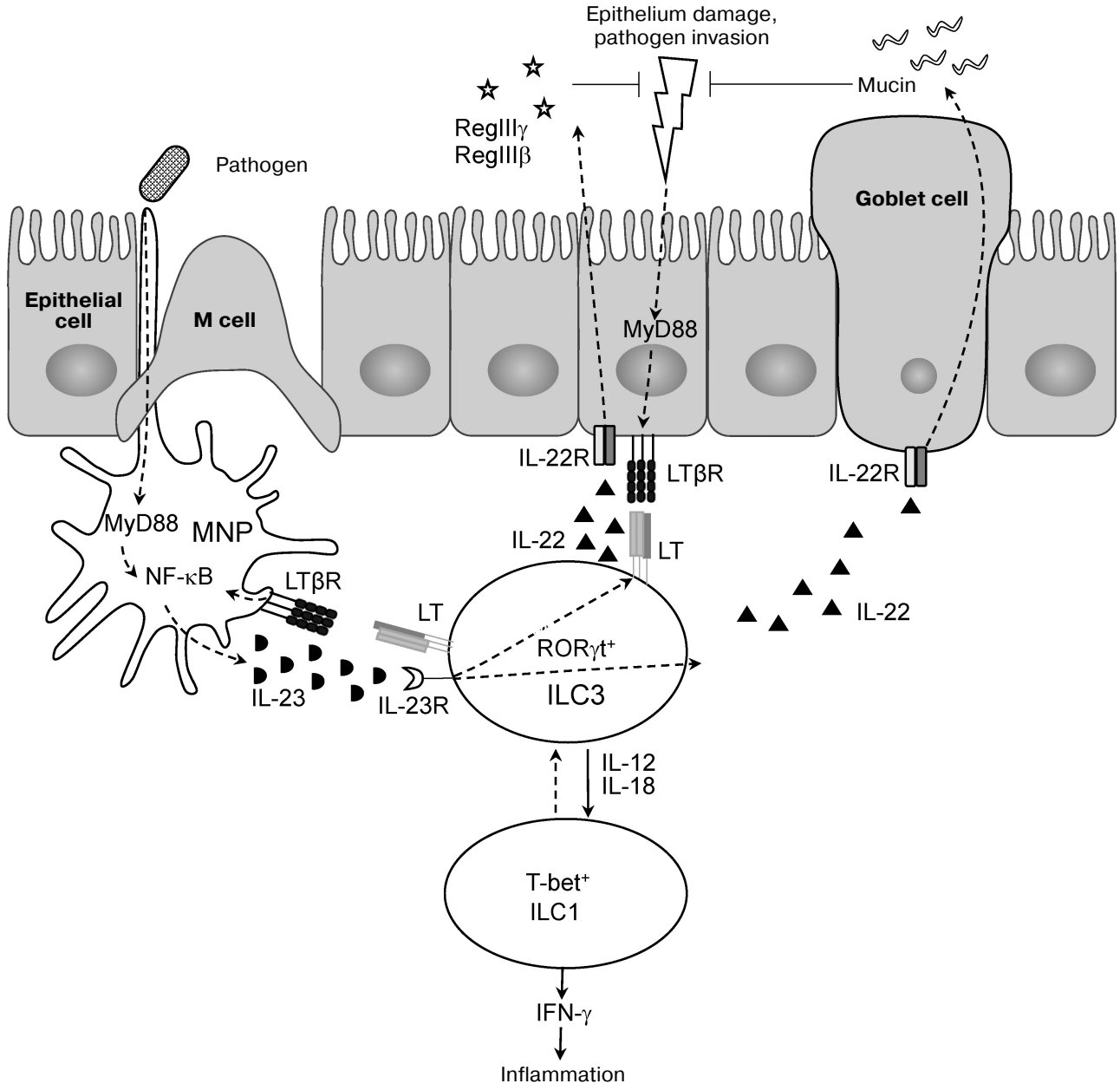

Fig. 2. Proposed mechanism of interaction of ILC3, mononuclear phagocytes (MNP), and epithelium upon mucosal damage. Epithelium damage results in penetration of pathogenic microorganisms deep inside tissues and MyD88-mediated signaling, which activates expression of NF-κB-dependent genes. Production of IL-23 by activated MNP (mainly by dendritic cells) stimulates production of IL-22 by ILC3s. In response to IL-22, epithelial cells proliferate and express antimicrobial peptides RegIIIγ and RegIIIβ, while goblet cells extensively excrete mucin. The described feedback loop between MNP and ILC3s, as well as LTβR-mediated interaction with epithelial cells, controls activity of pathogen at early stages of infection. Production of IL-12 and IL-18 by dendritic cells can cause differentiation of RORγt$^+$ ILC3 into pathogenic T-bet$^+$ ILC1 IFNγ-producing cells, which can aggravate inflammation.

of IBD are distinguished that differ in localization, etiology and symptoms: Crohn's disease and ulcerative colitis [27, 30-32, 130]. However, the pathogenesis of these diseases has a common pattern and consists in a combination of genetic susceptibility and certain environmental factors affecting the patient directly or indirectly involving their microbiota [27, 34, 131]. In a general case, impairment of the mucosal barrier function results in penetration of commensal microorganisms into tissues and activation of immune response. At this stage, genetic susceptibility to development of inadequate reaction to environmental factors may cause formation of continuous uncontrolled inflammation of the intestinal mucosa. An aberrant immune response includes activation of both innate and adaptive arms of the immune system with participation of proinflammatory cytokines, chemokines,

and transcription factors. Blocking the inflammatory process may inhibit disease development [35, 104]. Potentially, any molecule involved into initiation or progression of the disease may be considered as a target for therapy. Hypothetically, an ideal IBD treatment should induce and maintain continuous remission, which maximally reduces risk of complicated abdominal surgery. For a long time glucocorticosteroids, derivatives of 5-amino-

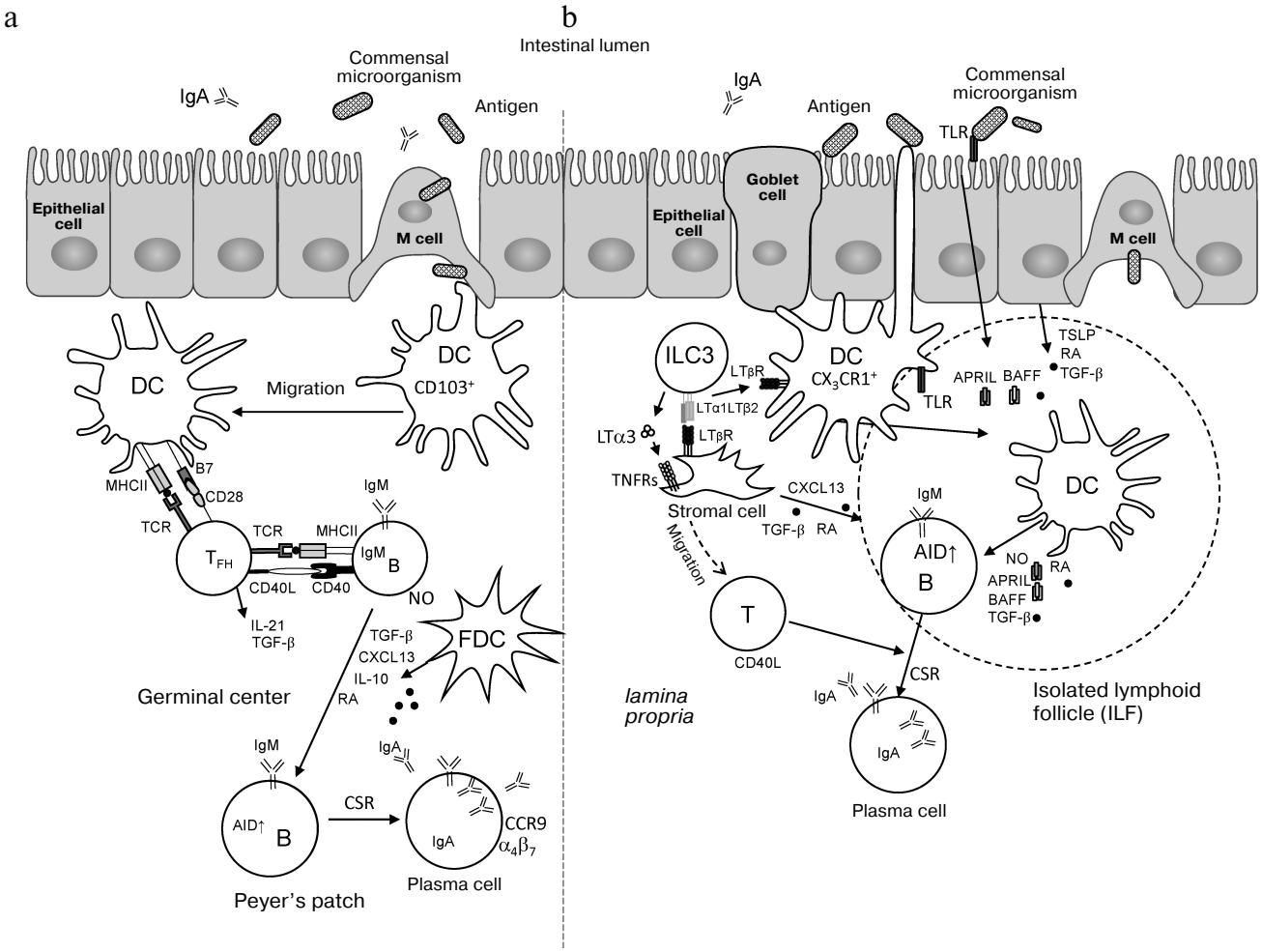

Fig. 3. Mechanisms of induction of IgA production in the intestine. a) T cell-dependent IgA induction in Peyer's patches. The single-layer epithelium associated with underlying B cell follicle (follicle-associated epithelium) contains approximately 10% of specialized microfold cells (M cells), which are able to capture antigens from the intestinal lumen and pass them to migrating dendritic cells ($CD103^+$ DC). Local non-migrating macrophage-like CX_3CR1^+ DCs can also directly capture antigens from the intestinal lumen by their long projections. Further, $CD103^+$ DCs migrate into interfollicular region of Peyer's patches for contact interactions and activation of naпve $CD4^+$ T cells. Then, $CD4^+$ T cells differentiate into follicular helper T cells (T_{FH}) under the action of retinoic acid (RA), TGFβ, IL-10, and thymic stromal lymphopoietin (TSLP) produced by epithelial cells and DCs. T_{FH} interact with antigen-specific B cells at the T–B region boundary and then migrate into B cell follicles following the CXCL13 gradient produced by follicular dendritic cells (FDC). CD40L, IL-21, and TGFβ produced by T_{FH} activate B cells and induce expression of activation-induced cytidine deaminase (AID), which is required for switching the immunoglobulin classes (CSR) to IgA. TGFβ, IL-10, RA, and nitric oxide (NO) produced by FDC and DC facilitate this process. In germinal centers, B cells increase expression of $\alpha_4\beta_7$ and CCR9 receptors in response to RA and migrate to the *lamina propria* upon interaction with high endothelial venules expressing MAdCAM-1. Further, B cells differentiate into plasmablasts and secrete polymeric IgA. During transfer through the epithelial cell layer, polymeric IgA is converted into secretory IgA (sIgA), which interacts with commensal microorganisms and neutralizes pathogens. LT with $ROR\gamma t^+$ ILC3 (LTi cells) are required for Peyer's patch development during embryogenesis. b) IgA induction in isolated lymphoid follicles (ILF) and in the *lamina propria*. CX_3CR1^+ DC capture antigen with transepithelial dendrites. $CD103^+$ DC can capture antigen from goblet cells. In ILF, DCs can also capture antigen from M cells. DCs are activated by signals from toll-like receptors (TLRs) and by TSLP and RA, produced by epithelial cells, and present antigen to B cells in ILF or in the *lamina propria*. B cells receive IgA-inducing signals from TNF superfamily cytokines – B cell activating factor of the TNF family (BAFF) and proliferation-inducing ligand (APRIL) – and from TGFβ and NO, which are produced by DCs and stromal cells. The latter are activated in turn by LT and TNF produced by $ROR\gamma t^+$ ILC3 cells. Interaction of BAFF and APRIL with BAFF-R, B-cell maturation antigen (BCMA), and transmembrane activator and CAML interactor (TACI) on B cells triggers expression of AID and formation of IgA-producing B cells within a lymphoid follicle. These B cells further differentiate into IgA-producing plasma cells in the *lamina propria*. As a result, special environment is created in the *lamina propria*, which is suitable for plasma cell survival. LT from $ROR\gamma t^+$ ILC3 is required for postnatal development of ILF. Membrane LT ($LT\alpha_1\beta_2$) from $ROR\gamma t^+$ ILC3 is required for T cell-independent induction of IgA production in the *lamina propria* by activating DCs. Soluble LT ($LT\alpha 3$) homotrimer secreted by $ROR\gamma t^+$ ILC is required for T cell-dependent IgA induction via T cell migration to the *lamina propria*.

Table 2. Characteristics of monoclonal anti-TNF antibodies approved by FDA (United States Food & Drug Administration) for IBD therapy

Antibody	Application	Source	Structure	Scheme	Half-life, days
Infliximab – IFX, Remicade	UC and CD, intravenously	chimeric (25% murine, 75% human parts) monoclonal antibody	Fab fragments are of murine origin, the rest is of human origin. Binds 2 TNF molecules		7-12
Adalimumab – ADA, Humira	UC and CD, subdermally	human monoclonal antibody	unique amino acid sequence. Binds 2 TNF molecules		10-20
Golimumab – GLM, Simponi	UC, subdermally	human monoclonal antibody	amino acid sequence is identical to that of Infliximab. Binds 2 TNF molecules		7-20
Certolizumab pegol – CZP, Cimzia	CD, subdermally	humanized monovalent compound	Fab-fragments covalently bound to polyethylene glycol. Fc domain is absent. Binds 1 TNF molecule		14

salicylic acid and immunosuppressant azathioprine possessing wide spectrum of contraindications and side effects were used as primary therapy for IBD [132, 133]. Emergence of TNF antagonists in the medical arsenal was a valuable addition to the existing methods for IBD therapy [133].

Increased levels of TNF in mucosa [134, 135], serum [134], and in stool [136] were found in IBD patients in comparison with healthy donors. Initially, TNF antagonists were introduced into broad practice during treatment of intestinal inflammation mainly as a second line therapy for patients who did not respond to conservative treatment. However, efficiency of TNF blockers as a first line therapy was soon shown [19, 137]. TNF antagonists have been approved by the Food and Drug Administration of USA (FDA) for IBD treatment: infliximab, adalimumab, golimumab, and certolizumab pegol [133, 138-140]. Their characteristics are given in Table 2. These drugs are also used for IBD treatment in Russia [31, 141].

The listed inhibitors are monoclonal anti-TNF IgG antibodies consisting of Fab-fragment, which binds antigen, and Fc-domain (or substituting molecule) connected to the Fab-fragment by a hinge linker [142].

All the monoclonal anti-TNF antibodies were designed for inhibition of TNF signaling. However, they have different efficiency, mechanism of action, contraindications, and side effects [140, 143, 144]. Though TNF inhibitors have revolutionized therapy of colitis, one third of patients do not respond to such treatment upon initial drug administration (primary non-responders), and up to 50% of patients who take anti-TNF antibodies eventually stop responding (secondary non-responders) [19, 145]. Therefore, understanding the mechanisms of the blocker action becomes essential for developing a successful therapeutic strategy.

In a number of *in vitro* studies, it was shown that differences in clinical efficacy of antibody might be related to its different affinity and avidity to soluble and membrane-bound TNF [146-148]. All TNF inhibitors based on monoclonal antibodies design efficiently bind and neutralize soluble TNF [146, 147, 149]. However, the ability of these blockers to neutralize membrane TNF showed contradictory results [146-150]. It is generally accepted though that these TNF inhibitors bind membrane fraction with lower affinity compared to the soluble TNF. As a result, efficiency of an inhibitor in the context of membrane TNF blocking depends not only on properties of the inhibitor itself, but also on levels of soluble TNF, which "competes" more successfully for antagonist binding [140]. This should be considered both by researchers when choosing the experimental colitis model and by physicians for selection of individual IBD therapy. It should be noted that in experimental colitis models in mice, selective inhibition of soluble TNF was sufficient to reduce inflammation. However, blocking both soluble and membrane-bound forms of TNF in mice was more efficient and accompanied by continuous stable remission [151].

In contrast to the above-mentioned TNF inhibitors, etanercept blocker (Enbrel) predominantly binds soluble TNF and is successfully used for treatment of rheumatoid arthritis. However, it was inefficient in treatment of IBD [152, 153]. Etanercept is an all-human chimeric protein consisting of two soluble TNFR2 moieties bound to the Fc-fragment of IgG1. It was suggested that etanercept has lower binding activity because of increased dissociation

rate (lower stability) of the complex with soluble and membrane-bound TNF [148]. Etanercept is also known to have lower ability to induce apoptosis in T cells, in contrast to adalimumab and infliximab, which may be a reason for low efficiency of etanercept in IBD therapy [19, 154]. Another potential explanation for limited efficiency of etanercept in IBD may be linked with its ability to block soluble $LT\alpha_3$ homotrimer [138], which is required for production of IgA and maintaining intestinal homeostasis [16].

The widespread use of anti-TNF therapy has led to an increase in number of registered systemic side effects, especially linked with infections, and inflammation in the skin and joints [138, 155]. One of the serious complications of anti-TNF therapy is activation of latent tuberculosis infection [156, 157]. Prognosis and risk management is complicated because in a number of cases it is hard to distinguish between side effects of anti-TNF therapy and non-intestinal manifestations of IBD. Taking into account that TNF blockers represent a fundamentally new class of therapeutic biological agents, fears about their long-term application safety required careful monitoring and fixation of all side effect cases [158, 159].

TNF antagonists are also efficient in treatment of severe forms of psoriasis [160]. However, continuous use of such therapy has revealed paradoxically high frequency of psoriasis development as a side effect [161]. Furthermore, cancellation of TNF blockers stopped the development psoriasis [162]. The mechanism of this reaction is not fully understood. Disruption of the balance between TNF and IFNα could be one of the reasons for development of psoriasis during anti-TNF therapy. It was shown that TNF blocks differentiation of plasmacytoid DCs (an IFNα source) from premature $CD34^+$ hematopoietic precursor cells *in vitro*. On the other hand, TNF inhibits secretion of IFNα by plasmacytoid DCs. Hence, neutralization of endogenous TNF increases the amount of secreted IFNα, which plays an important role in the psoriasis induction [163].

Beside psoriasis, anti-TNF therapy may cause such side effect as TNF alpha antagonist-induced lupus-like syndrome (TAILS) [164]. The etiology of this disease is not determined, although there are a number of hypotheses. According to one, anti-TNF therapy provokes massive apoptosis of proinflammatory immune cells accompanied by the release of DNA and other autoantigens, which results in antibody production and development of the lupus-like syndrome [165]. It should be noted that this mechanism is not applicable for the lupus-like syndrome caused by a course of etanercept, which does not induce apoptosis. A second hypothesis connects the disease pathogenesis with autoantibodies produced by continuously activated B cells, which are forced to handle frequent infections during immunosuppressive anti-TNF therapy [166]. According to another hypothesis, anti-TNF therapy activates humoral autoimmunity, thus shifting the Th1-Th2 helper responses [167]. A number of studies have demonstrated that application of TNF inhibitors might increase the risk of squamous cell carcinoma of the skin [168, 169]. On the other hand, emergence of skin cancer may be related to side effects of psoriasis and IBD therapy using ultraviolet A, psoralen [170], thiopurine [171], and other immunosuppressants.

Local inflammation at the injection site may be attributed to the side effects of anti-TNF therapy. A Danish research group measured levels of IgG and IgE antibodies against infliximab in the serum of patients after acute reaction to drug administration. They found that the presence of severe infusion reactions correlates with high levels of IgG, but not IgE antibodies. Thus, such a side effect is not a true anaphylactic reaction [172]. Finally, it was shown that the risk of bacterial, viral, and fungal infections is increased in patients with rheumatoid arthritis and IBD undergoing anti-TNF therapy [173, 174].

At present, blocking endogenous TNF is the most efficient IBD therapy. However, lack of a clear understanding of the antagonist action mechanisms and severe side effects determine an urgent need for further studies of TNF biology. Use of new mouse models such as "humanized" mice expressing human *TNF* genes as well as mice with tissue-specific TNF expression might be a valuable tool for studying the role of TNF in pathogenesis of intestinal inflammation and design of more efficient therapy for IBD [20, 175]. Other biologicals for IBD treatment are also being extensively developed and tested [25, 176, 177]. Similar to TNF, LT also plays a key role in regulation of the intestinal inflammation, but use of selective modulators of this cytokine in the IBD therapy is still poorly studied and requires additional research.

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