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Review article

Th9 lymphocytes and functions of interleukin 9 with the focus on IBD pathology

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ABSTRACT

The work presents the newest knowledge on a new phenotype of T helper lymphocytes (Th9) and on Interleukin 9 (IL-9). Processes leading to transformation of naïve T lymphocyte into Th9 lymphocytes are presented, including the role of IL-4 and TGFβ signaling. Involvement of transcription factor network in production of IL-9 is described. Other cells capable of expressing IL-9 and secreting IL-9 are portrayed. Diversity of IL-9 effects caused by activation of IL-9 receptors on various types of cells is presented. Principal effects of the activation of IL-9 receptor on T-cells seem to be antiapoptotic and stimulatory which leads to enhanced defense against parasitic infection and cancer development but, from the other side, it perpetuate chronic inflammation in autoimmune diseases and allergic processes. In the last years the role of IL-9 in autoimmune diseases such as rheumatic diseases and inflammatory bowel disease gained importance since the increased expression of this cytokine has been observed in animal models of intestinal inflammation and in groups of patients with ulcerative colitis. It was also noted that neutralization of IL-9 in animal models of ulcerative colitis leads to amelioration of inflammatory process, what could have significance in the treatment of this disease in humans in the future.

1. Introduction

Under the influence of certain compositions of cytokines naïve T helper lymphocytes are transformed into various functional T helper (Th) cells. After antigen presentation by antigen presenting cell (APC) and activation of T cell receptor in concert with co-stimulation by various cytokines, naïve T helper cell is transformed into either one of T helper cell types, which support inflammatory process or into regulatory T cell which mitigate the process and prevent the development of chronic inflammation [1]. Cytokines which are determining direction of T cell development in intestinal mucosa are secreted by dendritic cells, macrophages, epithelial cells and other immune cells already present in the intestinal wall. Phagocyting cells (macrophages and dendritic cells) are activated by foreign antigens which are detected by pattern recognition receptors (PRR). Antigens are phagocyted, processed and presented to T cells. Professional APC are secreting various sets of cytokines depending on the kind of stimulating antigens, among others, which in turn are influencing T cells to which antigen is presented in various modes [1].

For many years, a search for conditions under which naïve T helper cells are transformed into different types of active T helper cells has been carried out both in animals and in humans (*in vitro* experiments as well as *in vivo* studies). At first, based on the profiles of cytokines secreted, two types of Th lymphocytes have been distinguished: Th1 and Th2. For a long time, based on the presence of these T cells types in various pathological processes or the presence of cytokines secreted by them in the sites of pathology, theories on which type of Th is responsible for a given process have been developed. At present, Th1 is believed to be the major player in ulcerative colitis (UC) and Th2 in Crohn's disease (CD). Along with the development of more precise detection methods for cytokines, transcription factors, and genes encoding these cytokines additional phenotypes of Th have been distinguished: Th17, functional T helper lymphocytes (Tfh), regulatory T lymphocytes (Treg), and some years ago Th9. Combinations of activation factors (cytokines, their receptors, chemokines) which promote the development of naïve T cells into a certain Th type have been precisely determined both *in vitro* and *in vivo*. Detailed sequence of molecular intracellular events (activation of transcription factors, their interplay, epigenetic processes and activation of the genes) are under investigation and in spite of many controversies a picture of naïve T cell differentiation into various types of Th is emerging. It is now known that differentiation into different types is not the final fate of Th cells and, that if the milieu is changing the profile of cytokines secreted by the Th cell may also change. Therefore, a T cell of one phenotype will become

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a T cell of other type – this phenomenon has been named Th cells plasticity [2].

2. Review

2.1. Interleukin-9

Interleukin-9 (IL-9) was first purified in the seventies of the last century and described as possessing a T-cell and mast cell growth factor properties [3]. Later, human IL-9 gene was identified and described. IL-9 gene is located on chromosome 5, in the proximity of the IL-5/IL-13/IL-4 loci [4]. Interleukin IL-9 is a monomeric glycosylated polypeptide, consisting of 144 amino acids with molecular weight of 28–30 kDa and has a secretory signal sequence of 18 amino acids which belongs to the IL-7/IL-9 family of proteins [5,6].

At first IL-9 secretion has been described by Schmitt et al. [6] in activated murine T cells. They found that IL-9 is present in CD4+ T cells activated by IL-2 and its secretion is further enhanced by tumor growth factor-beta (TGFβ) and IL-4. They also found that IFN-gamma inhibits this process [5]. Initially, IL-9 secretion has been associated with Th2 cells phenotype, however, later it turned out that other T helper subsets are also capable of producing IL-9 [6].

Some years later it has been observed that TGF-beta added to T cell culture ‘reprograms' the naïve Th cells differentiation into Th2 phenotype to differentiation to other phenotype by suppressing the production of IL-4, IL-5 and IL-13 and promoting production of IL-9, IL-10 and IL-21. Finally, T helper lymphocytes which produced such set of cytokines where named Th9 cells. It has been observed that IL-4 signaling added to TGFβ signaling directed Th cell differentiation towards Th9 instead of Treg by inhibiting the ability of TGFβ to induce the expression of transcription factor FOXP3 (forkhead box P3) connected to Treg [6]. In many, sometimes very sophisticated experiments and observations, a network of cytokine receptors, their transcription factors and genes, which are responsible for transformation of naïve T helper cells into specialized type of T cells have been discovered and described, among them those responsible for Th-9 differentiation [6,7] (Fig. 1).

2.2. Factors promoting the development of Th-9 from Th-naïve cells and expression of IL-9 by T helper lymphocytes

At present there is a great amount of knowledge on the mechanism of action of cytokines which leads to IL-9 production and transformation of naïve Th into Th9 [7]. As in the case of other cytokines specific receptors, their signal traducers and activators of transcription of specific genes which lead to IL-9 expression have been found. The differentiation of T-helper-cell subsets is not only under the influence of specific sets od transcription factors binding to regulatory regions of genes, but is also affected by epigenetic processes [7]. In the last years it has also been found that microRNAs (miRNAs) may regulate the expression of genes that are necessary for the development, persistence and function of Th cells. Expression of IL-9 genes is being suppressed by miRNAs when IL-9 is overexpressed [8].

Transformation of naïve T helper cell into Th9 lymphocyte requires IL-4 and TGF beta are necessary [1,7]. Their signaling through signal transducer and activator of transcription 6 (STAT6), transcription factors PU.1, IRF4, GATA3 and BATF are essential [7,9]. From one side, gene expression which is inherited by progeny cells decides about the fate of the cell but at the same time this fate can be changed by altered environmental signals [9]. The transformation of CD4 naïve T lymphocytes into memory Th9 lymphocytes is associated with post-translational modifications of histones. Modified histones may change the structure of chromatin in such a way that promoter regions of genes involved in Th lymphocyte differentiation and secretion of cytokines are easier accessible [9]. It has been found that Th9-cell differentiation is determined by unique epigenetic modifications in the genes and especially the promoter region of transcription factor PU.1 which is of great importance for IL-9 synthesis [9].

Such complex mechanism of genes activation suggests that the expression of IL-9 depends not only on genes inherited by the individual but also on environmental factors, (intestine content, microbiome, history of nutrition, infections and other environmental factors). As mentioned above IL-4, IL-2 and TGF beta are principal factors in the process of Th9 development.

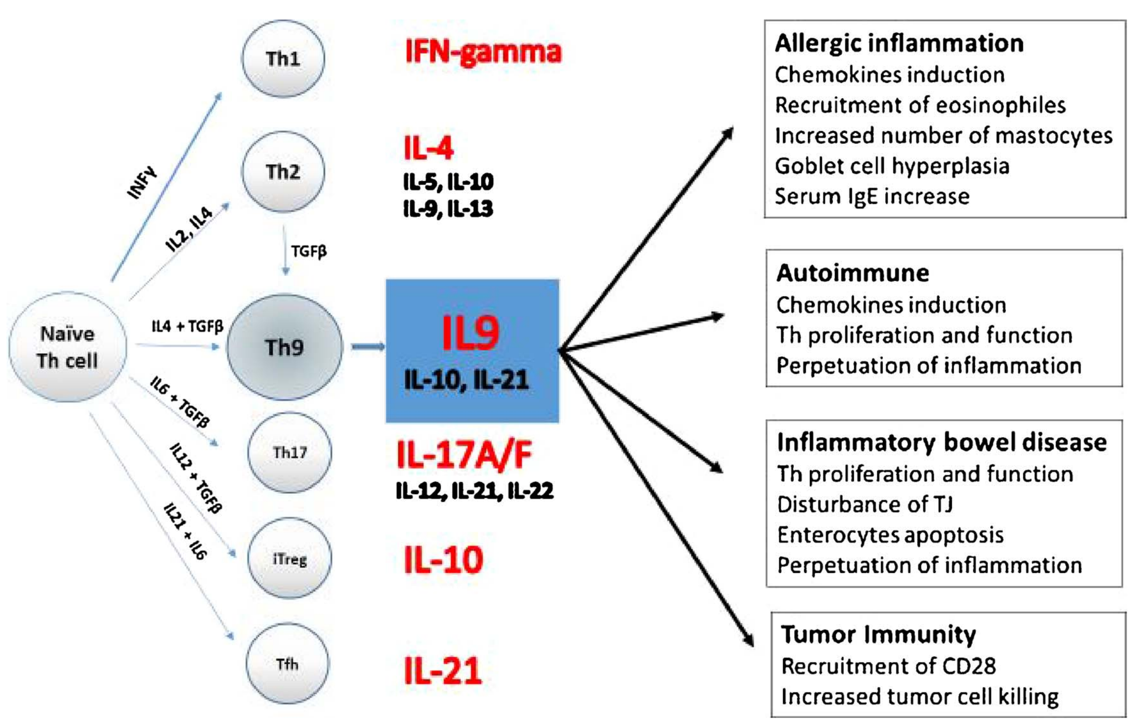

Fig. 1. T cell differentiation and Th9 and IL-9 functions. The development of T helper effector subsets from naive T cells precursors occurs under influence of certain sets of cytokines. IL-2, IL-4 and TGF-β signals promote Th9 development. Each type of Th cell secrets a set of cytokines which act on other cells. IL-9 effect is pluripotent and may influence pathological processes listed in frames. Based on references [6,7,9,11].

2.2.1. IL-4

STAT6 is the most important signaling transcription factor of the IL-4 receptor. STAT6 might not only bind the *Il9* locus directly and activate IL-9 production but can also suppress FOXP3 expression induced by TGFβ, and the expression of transcription factor T-bet, which is typical for Th1. Both these substances inhibit IL-9 production [10]. Other factor playing extremely important role is IRF4, which binds directly to the *IL9* promoter and increases *IL9* transcription. IRF4 induces *IL9* transcription and inhibits the expression of Th1 connected transcription factors that may block Th-9 differentiation, therefore, it promotes Th-9 cell development. B-cell-activating transcription factor (BATF) activates IL-9 transcription by binding to the promoter region of *Il9* similarly to IRF4, which additionally directs the transformation of naïve Th cell into the Th9 cell phenotype [5,9].

2.2.2. IL-2

Many years ago Schmitt et al. [5] demonstrated that production of IL-9 by CD4+ T cells is dependent on IL-2. IL-2 serves as a primary activator of STAT5 in T cells which binds within the IL-9 promoter and directly activate that promoter affecting IL-9 production. STAT5 also influences the production of IL-9 indirectly. Inhibition of IL-2 or STAT5 has been found to reduce the expression of transcription factors IRF4 and PU.1 which are required for differentiation of naïve T lymphocytes into Th9 lymphocytes.

2.2.3. TGFβ

As already mentioned TGFβ can transform Th2 into Th9. TGFβ activates a cascade of transcription factors: SMAD system and PU.1 which are fundamental for the expression of IL-9 [11]. Transcription factors such as STAT5 are necessary for development of IL-9 secreting T cells. IL-6 may influence STAT3, which in turn disturbs the capacity of STAT5 to induce the differentiation of T-cells [5].

Many, mainly in vitro experiments have been performed, that pointed to the influence of other cytokines which can be costimulatory agents in various types of IL-9 secreting cells, such as IL-1, IL-10, IL-25, IL-33 which increase the expression. IL-6, IL-21 and IFN-gamma inhibit IL-9 production [9].

2.3. Th-9 cells

Upon activation by APCs in the presence of IL-4 and TGF-beta, both naïve Th cells and Th2 cells are transformed into Th9 cells, which express interleukins IL-9 and IL-10 (Fig. 1). However, Th9 cells do not secrete IFN-gamma – the cytokine typical for Th1, or IL-4, IL-5, IL-13 – typical for Th2 or IL- 17 – typical for Th17. Transcription factors typical for other Th subsets such as: T-bet (Th1), GATA-3 (Th2), ROR-T (Th17), or FoxP3 (Treg) are virtually absent in Th9 cells but they express STAT6, IRF4, GATA3 and PU.1 which together with expression of IL-9 are markers of autonomous Th cell subset which is different from Th2 cell subset. However it is not completely certain if Th9 phenotype is stable or retain plasticity to acquire the potential to secrete other cytokines and become other Th types.

2.4. Other sources of IL-9

Th-9 is not the sole source of IL-9. This interleukin can also be produced by other Th cells (Th2, Th17, Treg), T cytotoxic cells (Tc), mastocytes and eosinophils. Cells which are not Th-9 can produce IL-9 permanently or transiently under the influence of certain environment. T helper type of cells is often determined exclusively on the basis of a set of cytokines produced after stimulation. This stimulation may, on one hand activate a particular gene which is already programmed for a particular cytokine or, on the other hand, cause the expression of the gene responsible for the production of a certain cytokine upon stimulation of the cell. The effects of the stimuli that cause IL-9 production are probably different from those which program *Il9* gene. A number of various transcription factors such as STAT5, STAT6, PU.1, BATF, IRF4, and Smad proteins are necessary for the programing of IL9 gene. Transcription factors listed above can also activate genes other than *Il9* and transform the structure of chromatin into the structure typical for mature and stable differentiated cells [9].

Other T helper types such as Th2, Th17, and regulatory T cells have been reported to produce IL-9, although generally in smaller amounts then Th9 cells [12]. In the presence of IL-4 and TGF-β, cytotoxic effector $CD8^+$ T cells are transformed into cells producing IL-9, which still possess low cytotoxic activity but which also express transcription factors T-bet and the CTL, typical for T helper cells. This type of cells were named Tc9. STAT6 and IRF4 are necessary for differentiation of Tc9 cells, similarly like Th9 [12].

Cells of innate immune system have also been observed to generate IL-9. Expression of IL-9 has been noted in mast cells after activation of toll like receptors (TLR) and other cytokines signals. Production of IL-9 by mast cells is increased in the presence of lipopolysaccharides [13]. Natural killer T (NKT) cells also produced IL-9 in certain experimental models. NKT cells which produced IL-9 have been demonstrated to protect mice against DSS-induced colitis [13].

2.5. IL-9 receptor (IL-9R)

The IL-9 receptor consists of two subunits: alpha chain (IL-9Rα) which is specific for this particular receptor and gamma chain, which is present in other cytokines' receptors (IL-2, IL-4, IL-7) [14]. The IL-9Rα belongs to the hematopoietin superfamily and has Box1 and Box2 motifs in its intracellular domain. After stimulation of IL-9R, signal transduction leads to STAT1, STAT3 and STAT5 activation which prevents apoptosis [15]. Activation of Box1 by IL-9 induces cell growth, activates STAT3 and induces certain genes' expression [16].

In general, activation of IL-9R leads to proliferation, prevention of apoptosis and activation of the cells possessing that receptor [6,7]. IL-9R is expressed on various activated T cell lines, mostly on Th2 and Th17, but is absent on naïve T cells. Therefore it can be expected that IL-9 would promote growth, differentiation and activation of these T cells and potentiate their action in various inflammatory processes [11].

IL-9R is also present on B cells and its activation increases class switching to IgE in these cells but also potentiates production of other immunoglobulins [17].

IL-9 R has also been discovered on neutrophils, mast cells, airway epithelial and muscle cells in the lungs of asthma patients as well as on mast cells and neutrophils in the lungs. IL-9 induces mast cell growth and provokes the accumulation of mast cell in various tissues [18].

Alpha chain of IL-9 receptor (IL-9Rα) is found on other, non-hematopoietic cells such as epithelial cells of the respiratory system, enterocytes, smooth muscle cells and keratinocytes. Chemokines production is stimulated by IL-9 in all of these cell types. As a result of this stimulation an increased mucus production by airway epithelial cells enhancement and changed barrier function in the intestinal tract have been observed. Thus, IL-9 seems to be of great importance for the function of various cells in inflammatory process [4,7,18].

2.6. Physiological and pathological effects of IL-9

IL-9, like many cytokines, exerts its activity through receptors found on a number of distinct cell types, therefore, Il-9 can influence diverse biological effects. Besides mast cells and T cells growth factor activity, as firstly described, IL-9 influences many other immune cells and tissue cells and can affect the development of inflammatory process [4,7]. IL-9, besides T and B lymphocytes, influences function of hematopoietic cells, mastocytes, eosinophils, smooth muscle cells and epithelial cells including intestinal mucosa.

2.6.1. Hematopoietic cells

A specific IL-9 alpha receptor chain (IL-9Rα) and gamma chain are present on hematopoietic cells. Activation of these chains by IL-9 regulates hematopoiesis. Together with IL-3, IL-9 activates the growth of IL-3-dependent cell lines and increases the synthesis of burst forming unit-erythroid (BFU-E) cells and erythroid colony forming unit (CFU-E) cells [9]. IL-9 provokes the proliferation of erythroid and myeloid cell lines [19] and also activates megakaryocyte progenitor cells [20].

2.6.2. Respiratory system: airway epithelial cells and airway smooth muscle cells

Effects of IL-9 on the airways can be of both direct and indirect character. Presence of IL-9 in the lungs has been reported to change genes' expression in epithelial cells [21] and to promote allergy through influence on IL-13 production [22]. IL-9 has multidirectional direct influence on airways epithelial cells and mucous cells as well as on airways smooth muscle cells all of which express IL-9 receptor [23]. Expression of IL-9 in the lungs has been demonstrated to result in airways inflammation, infiltration of eosinophils, hypertrophy of epithelial cells, exaggerated mucus production as well as elevated deposition of collagen in subepithelium [23].

2.6.3. Mast cells

Induction of mast cells growth and enhancement of their function was one of the first functions of IL-9 which have been described. IL-9 can affect mast cells progenitors growth in the bone marrow and the activity of mast cells [24]. In culture, IL-9, alone or in combination with receptor FcεRI, induces production of proteases and pro-allergic cytokines by mast cell [25]. From the other hand it has been demonstrated that in certain conditions mast cells are able to produce IL-9 and increase IgE mediated allergy [18]. In the study described above the authors identified mucosal mast cells which produced IL-9 and IL-13 which they termed mucosal mast cells producing IL-9 (MMC9). Antigen challenge was inducing activated MMC9. In patients with atopy and food allergy enhanced intestinal expression of IL-9 has been described [26].

2.6.4. Inflammation

One of the most prominent traits of IL-9 is anti-apoptotic activity and promotion of survival and proliferation of CD4+ T cells, mast cells, and other cells which can perpetuate chronic inflammation [6,7]. Although Th9 cells secrete IL-10 (inflammation regulatory cytokine) in high amounts no regulatory functions of these cells have been observed in inflammatory process. Many *in vivo* studies have confirmed lack of regulatory properties of Th9 under various conditions [7,27]. Some studies suggest that the anti-apoptotic property of IL-9 does not explain the whole activity of this cytokine and both *in vitro* and *in vivo* studies have shown that IL-9 stimulation results in the induction of production of various cytokines which can act as proinflammatory mediators [28,29].

2.6.5. Allergic inflammation

Th2 lymphocytes are the most important T cells in allergy development in which they orchestrate the immune response. At the beginning IL-9 has been linked to Th2 but it turned out that this cytokine is secreted by other types of T lymphocytes and that not only Th2 contributes to allergy. IL-9 is a growth factor for mastocytes and eosinophils, and it is also involved in the activation of B lymphocytes and Ig class switch to IgE [17]. IL-9 mRNA is highly expressed in T lymphocytes in bronchoalveolar lavage from asthmatic patients [30,31]. Some studies have indicated that IL-9 blockade may decrease allergic inflammation confirming a role of this cytokine in allergic process [31]. Blockade of IL-9 in a chronic lung inflammation has been shown to inhibit mastocytosis and airway remodeling. Infusion of anti-IL-9 antibodies in asthmatic mice has profoundly ameliorated the disease [31].

2.6.6. Parasitic infections

Closely related to allergic processes are the mechanisms defending against parasitic infections. Protective role of IL-9 in defense against intestinal parasitic infection has been described [32]. Increased expression of IL-9 in transgenic mice helped in removing of parasites after infection with *Trichuris muris* and *Trichinella spiralis* [33]. From the other hand injection of anti-IL-9 antibodies blocked mice immunity to *T. muris*. Similarly, the inhibition of the development of Th9 lymphocytes by interruption of TGF-β signaling pathway also caused weakened defense against *T. muris*. On the contrary, IL-9-deficient mice could overpower *Giardia lamblia* and *Nippostrongylus brasiliensis* infection, and application of IL-9 antibodies resulted in higher immunity to *Leishmania major*, due to the blockade of Th2 immunity [34].

2.7. Inflammatory diseases, autoimmune diseases

The IL-9R is present on many cell types. It has been found on such immune cells as T cells, B cells, lymphoid cells, mastocytes, neutrophils and such nonimmune cells as epithelial cells and smooth muscle cells. Therefore, IL-9 has an influence on a number of effector cells which participate in the regulation of inflammatory processes in many diseases. The engagement of the Th9 cells has been described in various types of inflammatory diseases, for example atopy, parasitic infections, as well as such autoimmune diseases as rheumatoid arthritis, vasculitis, lupus erythematosus, systemic sclerosis, autoimmune encephalomyelitis and ulcerative colitis [35–37].

2.7.1. Rheumatoid arthritis

IL-9 level has been significantly increased in the serum of patients with rheumatoid arthritis (RA), however, that increase seemed not to be related to disease duration [41]. Increased concentrations of IL-9 in the serum have also been observed in patients with asymptomatic RA and in relatives of those patients [38,39]. It has been noted that IL-9 levels were higher in the synovial fluid of patients with RA and that IL-9 promoted activity and proliferation of T cells in patients with rheumatoid arthritis[40].

Increased number of IL-9 secreting Th cells has been observed in the synovial fluid and peripheral blood in patients with psoriatic arthritis and it correlated significantly with disease activity [41]. Th9 cells and IL-9 have also been demonstrated to play a significant role in large-vessel vasculitis and systemic lupus erythematosus [42].

2.8. Inflammation of the intestine

2.8.1. Inflammatory bowel disease

Two main processes, among others, seem to play a fundamental role in the ethiopathogenesis of inflammatory bowel disease: disturbances in cellular immunity (both innate and adaptive) and impairment of mucosal epithelium integrity and function. Altered barrier function may allow antigens from the intestinal microflora to enter the mucosa and submucosa and induce antigen presentation and activation of T cells. In certain people, who might be genetically predisposed, the response to these antigens is excessive and the balance between anti- and pro-inflammatory immune responses moved towards chronic inflammatory process. However, the exact sequence of molecular events in inflammatory bowel disease is not known and, moreover, these sequence may be distinct in various groups of patients and in different variants of disease course. It is known that the activation of mucosal immune system may not only disturb intestinal epithelium but it also can influence intestinal microflora which can further perpetuate the process and it is not certain which of the components can be responsible for the onset of the disease [37,38]. IL-9 may affect both of those processes. Not only numerous cells of immune system are responsive to IL-9 but also intestinal epithelial cells, which in IBD overexpress IL-9R, are affected by IL-9 (6, 8). Therefore, it can be expected that IL-9 takes part in ethiopathogenesis of IBD [38].

Since Th9 cells along with IL-9 secret also IL-10, many authors speculated that they can have a regulatory effect and may be linked to tolerance and may ameliorate the inflammation. However, it turned out that in the classical colitis model Th9 cells initiated and further exacerbated the disease and the regulatory role of IL-10 has not been observed [13]. Th9 cells may incite intestinal inflammation but it is still unclear whether IL-9 promotes inflammation directly or by the influence on the production of other cytokines by Th1 or Th17 cells that can activate inflammatory process in the intestine. It has also been hypothesized that Th9 cells may acquire the capability of producing other proinflammatory cytokines [6,7].

IL-9 signaling between various immune cells is important in chronic mucosal inflammation − in the inflammation of the intestinal mucosa among others [43]. IL-9 has a regulatory role in inciting and perpetuating inflammatory responses in autoimmune diseases at mucosal surfaces. From various cells types with the membrane bound IL-9R, which are activated by IL-9, T helper cells are of special importance. IL-9 participates in activation and differentiation of these cells by activating the Jak/STAT pathway. Lately it has been suggested that inhibition of IL-9 signaling either by blocking of IL-9 secretion or by IL-9R blockade inhibits inflammatory process and may be efficient in the treatment of autoimmune and chronic inflammatory disorders including inflammatory bowel diseases, and allergic disorders for example food allergy and bronchial asthma in the future [44].

2.8.2. IL-9 and immune cells in IBD

In recent years several studies have been published, which addressed Th9 cells population, production and concentration of IL-9, expression of IL-9 receptors on potential target cells for IL-9, expression of the genes linked to IL-9 and its transcription factors in IBD.

Expression of adhesion molecules such as α4/β7 integrin on Th9 cells is high which permits these cells to re-enter the mucosa via interaction with their ligands (MAdCAM1) on gut endothelial cells and bind to E-cadherin on intestinal epithelial cells which causes that they may be abundantly present in the gut mucosa. Blocking of α4/β7 integrin with antibodies has led to the reduction of Th9 cells number in the colon [45]. Studies on cytokines genes regulation and expression of transcription factors in T cells in patients with UC have demonstrated an increased GATA-3 and IRF-4 expression in UC which supports the concept that Th2 and Th9 T cells may play the key role in ulcerative colitis [46]. Levels of mRNA of IL-9 and IRF4 have been increased in T cells from patients with active UC which was not detected in CD and may imply that Th9 cells may participate in the pathogenesis of UC rather than CD [47].

Experiments on animal models of colitis confirmed the importance of IL-9 in ulcerative colitis pathology. The TNBS-mediated colitis is associated with Th1 type cytokines and may simulate Crohn's disease inflammation and oxazolone-mediated colitis is associated with Th2 type cytokines and resemble human ulcerative colitis. Mice without IL-9 gene (IL-9 KO mice) are much less susceptible to all experimental colitides and are protected from inflammation [48].

Yuan et al. [44] investigated the presence of Th9 cells in tissues of mice with experimental UC. The authors also investigated the effect of anti-IL-9 antibody in the experimental DSS induced colitis in mice and demonstrated that Th9 cells expressing PU.1 were detected chiefly in the lamina propria and in smaller amounts in the intestinal mucosa intraepithelial lymphocytes. After anti-IL-9 antibodies were administered to animals the severity of inflammation in DSS induced colitis ameliorated what confirmed that IL-9 plays a role in the pathogenesis of UC.

Besides neutralizing antibodies, IL-9 production may be controlled by inhibiting regulatory transcription factors controlling IL-9 gene expression. In an elegant experiment Popp et al. [46] suppressed the production of IL-9 by the use of GATA3, specific DNAzyme in experimental colitis and mice did not develop inflammation. DNAzymes are able to penetrate into cells and may inhibit expression of various proinflammatory cytokines (including IL-9) what might be one of the treatment modes in UC in humans in the future [46,47].

It has been demonstrated that the count of IL-9 producing T cells in UC was elevated which was reasonable since the level of TGF-β, the inducer of IL9 production was also elevated in this disease [47]. IL-33 which may stimulate IL-9 production is present in higher amounts in UC but not in CD which can also confirm the theory that TGF-β together with IL-33 activate IL-9 production by T cells in UC [49].

Nalleweg et al. [50] studied the expression of IL-9 and IL-9R in peripheral blood, endoscopic biopsies and surgical samples from patients with UC. They observed that IL-9 was produced in high amounts by activated lymphocytes in the blood. Expression of IL-9 mRNA was significantly higher in samples from patients with UC than in healthy people, mostly in T lymphocytes and neutrophils. The authors have also observed that IL-9 was detected in the cells in which interferon regulatory factor 4 and PU.1, key Th9 lymphocytes transcription factors, were also overexpressed. IL-19R was overexpressed on circulating and gut polymorphonuclear leukocytes (PMN) as well as on gut epithelial cells. Stimulation of PMN with IL-9 enhanced IL-8 secretion and increased resistance of PMN to apoptosis.

Serum IL-9 concentration in IBD and its relation to severity of the disease has also been evaluated in some recent studies but outcomes of these studies were sometimes contradictory. Defendeti et al. [51] detected increased IL-9 concentration in serum from 41% of the patients with IBD and its level correlated with prognosis and IL-6 production in IBD. The authors found that there was a significant correlation between disease severity and IL-9 in the CD patients but not in the UC patients. Our recent study indicated that serum IL-9 level reflects endoscopic activity in UC and correlates with inflammatory indices [52].

2.8.3. Influence of IL-9 on gut epithelium in inflammatory bowel disease

It has been observed that expression of IL-9 receptors on enterocytes was elevated in IBD and that stimulation of IL-9R in gut epithelial cells induced STAT5 activation in these cells, which disturbed their growth and proliferation [50,51]. Lately the influence of various cytokines, IL-9 among them, on tight junction function and mucosa permeability has been discussed. It is well known that the number of tight junction strands in the epithelium of patients with Crohn's disease is reduced and that the proteins of tight junction differ from these of control group [48,53]. In ulcerative colitis, micro-erosions which are caused by intensified apoptosis in the intestinal epithelium are observed and the expression of claudin-2 is increased [54]. In the cited work the authors observed that the expression of claudin-2 was elevated in intercellular junctions (ICJ) in the samples of the intestinal wall from patients with both active CD and UC as compared with the samples from controls. Expression of zonulin ocludens-1 (ZO-1) in patients with CD and UC was reduced. This led to disturbances in the structure of TJs. This destruction could be the cause of altered permeability of the intestinal wall in patients with inflammatory bowel disease.

It is well known that interferon-gamma alone or in synergy with TNF-alpha may cause epithelial damage in Crohn's disease and change TJ structure which can lead to increased permeability. Interleukin-13 (IL-13), which is upregulated by IL-9, upregulates claudin-2 expression [55].

Gerlach et al. [60] in their excellent work describe how IL-9 and Th9 lymphocytes can participate in IBD pathogenesis by regulating intestinal barrier integrity. In their later paper Gerlach et al. [52] investigated the role of IL-9 in colitis. It turned out that lack of IL-9 in IL-9 knock out mice has ameliorated the severity of colitis. Occludin, sealing protein of tight junction, was expressed in higher amounts in IL-9 deficient organisms with colitis. In contrast, in IL-9 deficient animals the expression of claudin1 was reduced and claudin2 expression was similar in IL-9-defficient animal and in mice with IL-9. The authors drew a conclusion that influence of IL-9 on tight junction is complex and pleiotropic and that this cytokine may influence regulation of intestinal permeability in colitis [52].

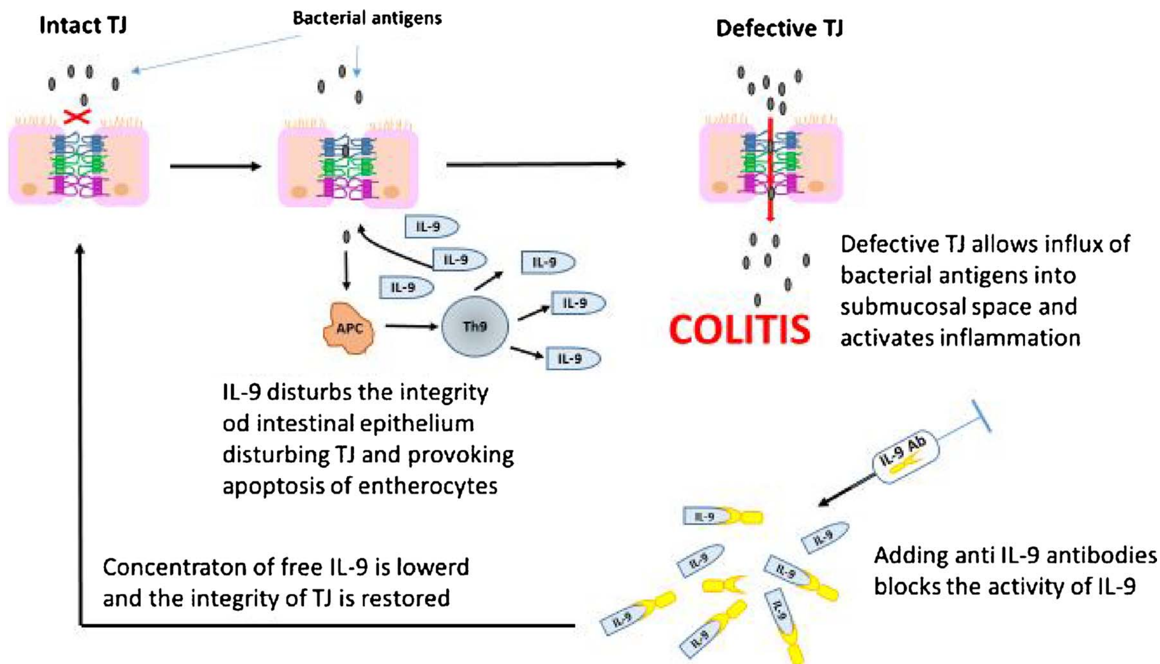

Fig. 2. Possible involvement of IL-9 and anti-IL-9 antibody in the regulation of intestinal mucosa integrity in ulcerative colitis. Based on references [6,47,50,56].

In culture systems besides regulation of intracellular TJ, IL-9 reduced proliferation of epithelial cells and induced their apoptosis, which is in contrast with T-cells where IL-9 exerts anti-apoptotic activity [55]. Therefore, IL-9 is able to influence the function of intestinal epithelial cells and may impair barrier function in colitis which will permit the translocation of commensal and harmful bacteria into the intestinal wall [47,56]. A possible involvement of IL-9 and antibody anti-IL-9 in regulation of intestinal mucosa integrity in ulcerative colitis is shown on Fig. 2.

3. Conclusions

A significant number of reports confirming important role of IL-9 in ulcerative colitis has been published. The number of T cells which are expressing both IL-9 and its transcription factor PU.1 is elevated in patients with ulcerative colitis in comparison to controls. Severity of acute and chronic colitis is ameliorated in IL-9 deficiency and the absence of transcription factor PU.1 in T lymphocyte protects from colitis. Experimental treatment with neutralizing antibody against IL-9, and metabolites suppressing IL-9 genes and transcription factors suppressed colitis. In contrast, IL-9 infusion or Th9 transfer exacerbated experimental colitis. At the cytological level IL-9 impaired intestinal barrier function directly influencing TJ proteins and enterocytes which also can explain its role in colitis.

Conflict of interests

The authors declare no conflict of interests

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