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ABSTRACT

Diabetic foot is one of the serious complications of diabetes, which formed foot ulcer in the later stage of diabetes mellitus. The treatment of diabetic foot ulcer is difficult, greatly increasing the risk of amputation, which will seriously affect the quality of life of patients. Promoting the healing of ulcer in the early stage has become the guiding ideology for the treatment of diabetic foot ulcer. Macrophages, as important immune cells, play a regulatory role throughout the healing process of diabetic foot ulcers. Therefore, we can provide a new direction for the treatment of diabetic foot ulcers by deeply understanding the mechanism of macrophages and the healing of diabetic foot ulcers.

1. Introduction

Diabetic foot, one of the most serious complications in the late stage of diabetes, has become the main cause of treatment, disability and death for diabetic patients[1]. Diabetic foot as one of the common diseases of peripheral blood vessels which characterized by foot infection or ulcer as far as deep tissue destruction due to nerve abnormality and vascular disease in the distal extremity[2]. Diabetes is one of the four major chronic non-communicable diseases. Epidemiology shows that about 387 million adults worldwide have been diagnosed with diabetes[3], 15%–25% of patients with diabetes will develop diabetic foot. About 40% of lower limb amputations in adults are due to diabetes[5], in which 85% of lower limb amputations caused by diabetes foot ulcers[6]. Diabetic foot ulcers (DFU) are often difficult to heal due to lower limb neurovascular abnormalities, local microcirculation and oxygen metabolism disorders. The incidence of diabetic foot ulcers in diabetic patients is 4%–10%[7]. Promoting ulcer healing, preventing secondary infection and recurrence, and reducing amputation have become the main targets of diabetic foot treatment[7]. Wound healing process includes three stages: inflammatory reaction stage, granulation tissue hyperplasia stage and scar formation stage. In this process, many cytokines and inflammatory factors are required to participate together. Although the specific mechanism of difficult healing of diabetic foot ulcers is still unclear, some studies have pointed out that the delayed healing of diabetic foot ulcers is closely related to the delay and imbalance of inflammatory response[8]. Macrophages, as an important immune cell, play an important role in clearing pathogens, mediating inflammatory response, tissue remodeling and repair when the body is damaged[9,10]. In recent years, it has been found that macrophages play an important role in the healing process of diabetic foot ulcers. This article will give an overview of them.

2. Overview of macrophages

2.1 Origin of macrophages

As an important component of the monocyte phagocytosis system (MPS), macrophages are widely distributed in various tissues and organs of the body. According to the latest research, most of the macrophages come from monocytes in the blood, while others come from hematopoietic stem cells in the original hematopoietic tissue and fetal liver monocytes[11–13]. Mononuclear cells are gradually developed and differentiated into peripheral blood from the oriented stem cells in bone marrow. Then monocytes enter the tissue through the walls of the blood vessels and further develop into macrophages. In this process, the phagocytic capacity of cells was gradually enhanced and secreted a large number of soluble factors[14,15].
2.2 Macrophage polarization

Due to the strong plasticity of macrophages, macrophages can adapt to different microenvironments by changing different phenotypes, thus exerting different functions[16]. Under the stimulus of different pathogen-associated molecular patterns PAMP and damage-related molecular patterns damps, macrophages polarized into two types: M1 macrophages and M2 macrophages or macrophages with classical activation pathway and macrophages with alternative activation pathway[17]. M1 macrophages are polarized under the induction of bacterial products such as lipopolysaccharide (LPS), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and granulocyte macrophage colony stimulating factor (GM-CSF) [18]. M1 macrophages are characterized by high expression of CD86, MHC II and CCR2, secretion of high levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin, IL-1, IL-16, IL-12 and nitric oxide pro-inflammatory mediators. The antigen presenting ability and bactericidal ability of M1 macrophages were improved. In addition, M1 macrophages play the role of clearing pathogens by regulating and promoting the immune responses of Th1 and Th17. However, in this process, normal body tissues may also be destroyed by M1 type macrophages, so M1 type macrophages are also called inflammatory macrophages[15,19]. M2 macrophages can be divided into M2a, M2b and M2c subtypes according to their functions. The M2a induced by IL-4 and IL-13 is characterized by high mannose receptor expression and enhanced endocytosis, and has the functions of promoting cell growth, tissue repair and Th2 immunity. The M2b stimulated by immune complex, lipopolysaccharide or interleukin -1 is mainly characterized by high secretion of inflammatory cytokines TNF-α, IL-6, IL-1 and anti-inflammatory factor IL-10, and low secretion of IL-12, and has the functions of inhibiting acute inflammatory reaction and promoting Th2 immunity and humoral immunity. The M2c induced by glucocorticoid, IL-10 and TGF-β is characterized by high secretion of IL-10, protease and low expression of MCHII molecules, and has the functions of inhibiting inflammatory reaction and promoting tissue remodeling[14,20,21]. M1 macrophages appear at the early stage of wound healing, which play a role in promoting wound inflammation, strengthening wound necrosis tissue and cell clearance, and preventing bacterial invasion by secreting inflammatory factors and inflammatory chemokines[22]. At the end of the inflammatory reaction, the number of local macrophages in the wound gradually decreased, and the remaining macrophages eventually turned into M2-type macrophages. The expression level of inflammatory factors secreted by M1 macrophages also gradually decreased. At this time, the secretion of anti-inflammatory factors will increase, the inflammatory reaction of the wound surface will be reduced, and the local cells of the wound surface will be proliferated and vascularized[23].

2.3 Polarization of macrophages in diabetic foot ulcer

As diabetes patients are in a state of high glucose, changes in the local microenvironment of ulcers, coupled with ischemia and hypoxia, cause abnormalities in the phenotype of macrophages, such as an increase in the number and proportion of M1 macrophages[24,25]. The level of inflammatory factors secreted by macrophages also increased. However, their ability to express and phagocytize inflammatory factors after stimulation and activation decreased significantly.

3. Macrophages in local wounds of diabetic mice

In the study of chronic skin ulcer wounds in diabetic mice, the wounds in the control group were smaller than those in db/db diabetic mice after 7 d of treatment. PCR detection of the relative expression of M1 and M2 cytokine mRNA in wound surface showed that the contents of M1 macrophage cytokines IL-12 and TNF-α in the db/db mice model of type II diabetes were higher than those in the normal group, while the contents of M2 macrophage cytokines VEGF and TGF-β were lower than those in the normal group. Therefore, it is presumed that the number of inflammatory macrophages in the wound surface of db/db diabetic mice increases and promotes the secretion of inflammatory factors. Due to the increase of inflammatory factors in the wound surface, the transformation from M1 type macrophages to M2 type macrophages is blocked, resulting in excessive inflammatory reaction of the wound surface and delaying the healing of the wound[26]. In a study, it was found that the number of inflammatory cells in the wound tissue of diabetic mice was small on the 3rd day after the model was established, and the contents of TNF-α and IL-6 in the wound tissue were also significantly lower than those in the blank group, indicating that the ability of diabetic mice to deal with traumatic stress was reduced. On the 6th day after modeling, the contents of TNF-α and IL-6 in serum and wound tissue of diabetic mice increased sharply. On the 12th day after wound modeling, inflammatory cells in the wound tissue of diabetic mice were still significantly infiltrated, the density of macrophage immunohistochemical marker CD68 positive cells was high, and TNF-α and IL-6 in serum and wound tissue were still at a high level. The above results showed that the inflammatory reaction of the wound surface of diabetic mice was slow and the infiltration time of inflammatory cells was longer in the later stage[27]. In a study on inoculation of diabetic mice with Pseudomonas aeruginosa, M1 macrophage marker tumor necrosis factor-α (TNF-α), IL-6 and IL-1b were highly expressed in the wound of diabetic mice infected with Pseudomonas aeruginosa on the fifth day compared with the control group, and M2 macrophage marker proinflammatory factor IL-10 was also up-regulated and kept at a high level on the fifth to ninth days after the model was established. The results showed that Pseudomonas aeruginosa promoted M1 and M2 phenotypic activation, especially M1[28]. Since Pseudomonas aeruginosa can produce bacteria, which are the main components of the outer membrane of lipopolysaccharide gram-negative bacteria, LPS acts as proto - endotoxin, and combines with TLR 4 receptor complex in macrophages to promote the secretion of pro-inflammatory cytokines[29]. In another diabetic mouse ulcer experiment, it was found that congenital immune cells such as Gr - 1 neutrophil marker and Ym1 macrophage were delayed in the wound, and the expression of M2 macrophage-related genes Ym1 and arginase 1 in the wound was significantly reduced. Through PCR array analysis, the expression of cytokines in the wound of diabetic mice was changed, mainly characterized by high levels of interleukin (IL)-17 and IL-20 mRNA[30].
3.1 Macrophages in local wounds of diabetic foot ulcer patients

In a study of local pathological sections of 259 diabetic foot patients, a large number of CD68+ macrophages were found in the dermis[31]. In addition, in a comparison of macrophage expression in local tissues of diabetic foot ulcer, non-diabetic foot ulcer and normal control group, it was found that the level of macrophage expression in epidermal tissue was higher than that in the other two groups, while the level of macrophage expression in dermal tissue was highest in diabetic foot ulcer group, followed by non-diabetic foot ulcer group. The expression level of VEGF-C in diabetic foot ulcer group was lower than that in the other two groups, indicating that the number of macrophages in diabetic foot ulcer tissue did not increase significantly, the ability to secrete VEGF-C decreased, and there was an insufficient inflammatory response[32].

3.2 Drug intervention on macrophages to promote healing of diabetic foot ulcer

Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) is a glycoprotein secreted by activated T lymphocytes, macrophages, endothelial cells and fibroblasts, and has been widely used in clinic. RhGM-CSF promotes wound healing mainly by increasing the activity of neutrophils and circulating macrophages. Relevant studies have pointed out that the cure rate of subcutaneous injection of rhGM-CSF around the wound surface of early diabetic foot ulcer patients is 53.9 %, 66.7 % after one month of treatment, and the total effective rate of local application of rhGM-CSF is 82.35 %[33-35].

4. Related mechanisms mediate macrophages to regulate diabetic foot ulcer healing

4.1 Epigenetic regulation of macrophage effect on diabetic foot ulcer

Epigenetic modification regulates gene transcription by altering chromosome structure through DNA methylation, histone modification and non-coding RNA. With the change of tissue microenvironment, epigenetic modification adaptively exposes or hides related domains, thus affecting the formation of transcription complexes, and finally regulating gene expression products to adapt to the change of microenvironment[37]. The high blood sugar level of diabetic patients has led to changes in the microenvironment in vivo, further changing the epigenetic mechanism, and eventually leading to the occurrence of diabetic complications[38].

DNA methylation plays a role through methylation of cytosine residues on CpG (cytosine - phosphate - guanine) dinucleotides and catalysis of DNA methyltransferase (DNMTs). Related studies suggest that high sugar microenvironment is conducive to methylation of CpG dinucleotides. DNMTs, as a redox sensitive enzyme, is susceptible to superoxide and free radical species in diabetic high sugar environment, inducing hypermethylation of some genes, further leading to inhibition of early response genes[39].

Histone methylation, as an important epigenetic marker, is mediated by histone lysine methyltransferase (HMTs) to catalyze methyl transfer from S-adenosylmethionine (SAM) to the basic amino acid of histone amino terminal domain[40]. The protein family containing SET (the set - domain containing proteins) is a member of histone methyltransferase. Under the condition of high glucose in diabetes mellitus, SET enzyme activity changes. Transient hyperglycemia can induce Set7 enzyme change profile to change methylation of histone 3lysine 4 (H3k4) at NF-κB promoter site, thus causing high expression of inflammatory markers such as monocyte chemoattractant protein 1 (MCP - 1) and vascular adhesion molecule 1 (VCAM - 1)[41].

The enzymes involved in histone demethylation mainly include lysine-specific demethylase (LSDs) and demethylase containing (JMD). Related studies show that JMD3 in demethylase not only has a regulatory effect on IRF-4, which causes macrophages to polarize into M2 type, but also can cause M1 type macrophages to overexpress under high glucose conditions[42]. On the basis of the above research, a series of drugs with anti-diabetic activity and anti-oxidation effect, such as 3 - hydroxyflavone, coumarin analogues and other synthetic molecules: gamma - benzopyranone analogues and thiazolidine - 4 - one derivatives, have been developed on diabetes treatment drugs[43-45].

4.2 NF-κB signaling pathway regulates macrophage action on diabetic foot ulcer

Nuclear transcription factor kappaB (NF-κB), as an important transcription regulator, plays an important role in TNF - α synthesis[46]. The location of NF - κB p65 subunit in the original 264.7 cell is a marker of NF - κB activation. In hyperglycemia, NF - κB activation is in a continuous state, and the synthesis of advanced glycosylation end products (AGEs) is accelerated, resulting in ACGs not being cleared in time, accumulating in vivo and damaging cell tissues[47].

The 7nicotinic acetylcholine receptor (7nAChR) is a classical neuronal receptor that is widely expressed in infiltrating macrophages[48]. In a study, it was found that a large number of inflammatory cells infiltrated the wound surface of normal control mice on the 1st and 3rd days after the model was established, a large number of fibroblasts (FBCs) grew on the 5th to 14th days, and good connective tissue was formed on the 21st day. A small amount of inflammatory cells infiltrated the wound surface of diabetic mice on the 1st and 3rd days after the model was established, and the number of inflammatory cells increased significantly after 5 days, sparse connective tissue was found locally on the 21st day after the model was established, and TNF - α level and AGEs level of the wound surface of diabetic mice increased significantly on the 5th day after the model was established. The diabetic mice in the 7nAChR receptor selective agonist intervention group took PNU282987 once a day from the 5th day to the 14th day after the model was established. The diabetic mice in the PNU282987 group had a reduced number of macrophages on the wound surface of diabetic mice on the 21st day after the model was established. The control group, the number of fibroblasts and collagen deposition were significantly reduced. Advanced Glicationen Products (AGEs) stimulate NF - κB p65 translocation from cytoplasm to nucleus and increase NF - κB activation. AGEs - induced NF - κB activation was blocked when pnu 282987 was applied. The results show that the TNF-α level in the wound surface was lower than that in the diabetes group. Compared with the control group, the number of fibroblasts and collagen deposition were significantly reduced. Advanced Glicationen Products (AGEs) stimulate NF - κB p65 translocation from cytoplasm to nucleus and increase NF - κB p65 DNA binding activity. AGEs - induced NF - κB activation was blocked when PNU282987 was applied. The results show that pnu 282987 reduces TNF - α production by inhibiting AGEs and reducing its stimulation to macrophages. Pnu 282987 also significantly inhibited AGE - induced activation of NF - κB and RAGE expression in macrophages. On the basis of the above research, activation of 7nAChR can become an effective way to inhibit AGE - induced TNF - α production by blocking pro-inflammatory RAGE / NF - κB signaling pathway[49].
4.3 Notch signal pathway regulates macrophage action on diabetic foot ulcer

Notch signal is a key regulator of macrophage biological function, including 4 Notch receptors (Notch1-4) and 5 ligands (Delta1, 3, 4, Jagged1-2), in which Notch4 expression is limited to mature macrophages, pancreas and epithelial cells[50]. In a study using flow cytometry to detect the dynamic changes of Notch receptor in diabetic rats, it was found that the expressions of Notch1, Notch2 and DLL4 were significantly higher on the 3rd d of modeling than on the 1st day, and the Hes1 gene was also increased on the 3rd day. The above results indicate that Notch signal is dynamic in macrophages during wound healing, and may play a role in macrophage function in the early stage of inflammation[51]. In addition, DNAMamloxedlyz2Cre + mice with suppressed Notch signal had a decrease in the number of macrophages in the early wound surface and delayed wound healing. DNAMAML1loxP-Lyz2Cre+ mice had less re-epithelialization and granulation tissue formation at the wound edge. Tricolor staining showed that DNMAML1loxP-Lyz2Cre+ mice had less collagen deposition. Compared with the control mice in the same nest, the IL -1β and TNF in DNAMAML1loxP-Lyz2Cre+ macrophages were significantly decreased. Related experiments show that inhibition of Notch signal leads to reduction of inflammatory cytokines produced by macrophages, which also proves that Notch plays a role in regulating wound macrophage phenotype and inflammation in vivo, and Notch signal changes in diabetic wound macrophages.

In addition, some studies have pointed out that Notch signal may participate in the formation of M1 type macrophages through some epigenetic mechanism. Expression of Notch-1 in macrophages is regulated by LPS, which activates the expression of genes Hes1 and Deltex downstream of Notch through MyD88 - dependent or non-dependent pathways. When RBP-Jκ is eliminated and Notch signaling pathway of macrophages is blocked, when LPS, TNF-α and other M1 - type inducing factors are given, macrophages cannot polarize to M1 - type but to M2 - type. However, after activating Notch signaling pathway, macrophages polarized to M1 type regardless of M1 type or M2 type inducing factors. Blocking Notch signaling pathway of macrophages with GSI can reduce the expression of M1 macrophage-related factors, while the expression of MHC - II tends to be up – regulated[52]. Activation of Notch ligands DLL-1, DLL-4 and their target genes Hes1, Deltex will promote the activation of macrophages. In addition, Notch signaling pathway can also indirectly regulate macrophage development by regulating the expression of macrophage surface marker CD11b[53].

5. Discussion

Diabetic foot ulcer, as a chronic refractory ulcer, its treatment has become the research focus of relevant scholars. The diabetic foot ulcer is located in a high glucose microenvironment for a long time, which leads to the decrease of macrophage stress to wound. The early inflammatory reaction is relatively slow and lasts for a long time, presenting a state of continuous inflammatory reaction locally, and delaying the wound healing process. Therefore, it has become the starting point for the treatment of diabetic foot ulcer to regulate local inflammatory reaction and prevent the extension of inflammatory reaction.

Macrophages, as important specific immune cells, have attracted much attention in the research of wound healing process. In addition, macrophages also play an important role in the occurrence and progression of diabetes and diabetic ulcer. Macrophages can be polarized into M1 - type macrophages and M2 - type macrophages. M1 - type macrophages mainly promote inflammatory reactions, while M2 - type macrophages exert inhibitory effects on inflammatory reactions[22,23]. Therefore, adjusting the balance of M1 and M2 macrophage levels is the key point to regulate the inflammatory response and prevent the inflammatory response from being too long. The difficulty in healing diabetic foot ulcers is partly due to the prolonged inflammatory reaction. Correspondingly, if the polarization of macrophages can be relatively inhibited to M1 type and the polarization is promoted to M2 type, the local inflammatory reaction of diabetic foot ulcers can be effectively shortened to achieve the effect of promoting wound healing. In addition, recent studies have found that macrophages are regulated and interfered by epigenetic modification, NF-κB signaling pathway, Notch signaling pathway and other mechanisms during ulcer healing. Epigenetic modification mainly makes M1 macrophages highly expressed by DNA methylated demethylase Jmjd3 in high sugar state, so histone demethylase inhibitor may be a new treatment option for diabetic ulcer[42]. Under hyperglycemia, NF-κB activation is in a continuous state, which accelerates AGEs synthesis and accumulation in the body, induces local TNF-α level to increase and hinders wound healing. Therefore, treatment can begin with improving the continuous activation state of NF - κB[47]. In addition, Notch signal mainly plays a regulatory role on macrophages in the early stage of inflammation. Relevant studies have pointed out that inhibition of Notch signal can reduce IL-1β and TNF-α secreted by M1 - type macrophages, thus reducing the level of inflammatory response. Therefore, inhibition of Notch pathway will also become a direction for the treatment of diabetic ulcer[51]. Therefore, further clarifying the polarization mechanism of macrophages and grasping the best intervention time can provide the best treatment plan for the treatment of diabetic foot ulcer, which will become the direction of our future efforts.

References