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Research progress of CpG-DNA in regulation of immune system and signal pathway

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ABSTRACT

CpG-DNA is a DNA sequence with immunostimulatory function as the core of unmethylated CpG motif. It includes synthetic oligodeoxynucleotides containing CpG motifs and genomic DNA of lower organisms such as bacteria, viruses, and invertebrates. CpG-DNA as a potential immunostimulatory factor can be recognized by the vertebrate natural immune system, rapid activation of natural immune response. It can trigger a protective immune response to remove foreign pathogens. CpG-DNA activated cell signaling mechanisms include endocytosis of CpG-DNA, specificity with Toll-like receptor 9 and a series of signal cascades that induce the expression of target genes and Feedback by various endogenous factors. This paper reviews the basic concepts of CpG-DNA, the characteristics of TLR9 receptors, the regulation of the immune system and its signaling pathways.

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Abbreviations: CpG-DNA, DNA sequence with the unmethylated CpG motif; TLR,Toll-like receptor; BCG, Bacillus Calmette-Guérin; ODNs, deoxyribonucleotides

Introduction

The DNA sequence with the unmethylated CpG motif as the core activates the cells containing Toll-like receptor 9. CpG-DNA induces an immune response characterized by the production of Th1 and proinflammatory cytokines, acting as a vaccine adjuvant in many drug clinical trials. This paper reviews the basic concepts of CpG-DNA, the characteristics of TLR9 receptors, and the regulation of CpG-DNA on the immune system and its signaling pathways.

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1. The concept of CpG-DNA

The unmethylated CpG DNA in the microorganism has an immunostimulatory effect that directly or indirectly activates the immune system and enhances the immune response. BCG-CpG-DNA is a genomic DNA fragment extracted from BCG, rich in unmethylated CpG motif[1]. Whether the bacterial DNA has an immune stimulating effect on the mammalian immune system depends on the amount of unmethylated CpG dinucleotide in the bacterial DNA. Mammalian DNA is mostly methylated CpG dinucleotide, so mammalian DNA does not have immune stimulating effect[2].

The synthetic single-chain unmethylat--ed CpG deoxyribonucleotides (ODNs) can mimic the immunostimulatory effects of unmethylated CpG-DNA in microbial cells. According to the specific sequence composition and immune function, the synthetic CpG-ODN can be divided into four categories, namely, class A, class B, class C and class P, according to different in vitro test needs, for the replacement of unmethylated cells CpG-DNA. Class A CpG-ODN consists essentially of chimeric phosphorothioate and phosphodiester skeletons, polyglycine palates at the 3 'or 5' end containing at least one unmethylated CpG dinucleotide, Mainly used to stimulate dendritic cells to produce interferon and thus activate the natural killer cells. Class B CpG-ODN is mainly composed of thiophosphate skeleton, and a number of un--methylated CpG motifs are present between the skeletons, mainly for stimulating the proliferation and differentiation of B cells. And the 5 'end of C-type CpG-ODN Methylated CpG motif hexamer, 3 'end with rich GC palindromic sequence structure, function at the same time with Class A and B type ODN characteristics [3]. P type is similar to the C type, but the stimulation of DC cells is slightly stronger than the role of B cells [4].

2. CpG-DNA receptor

CpG-DNA is an agonist of Toll-like receptor-9 (TLR9), and its immuno--modulatory effect is thought to be achieved by direct or indirect activation of TLR9-expressing cells. In humans, TLR9 is mainly expressed in B cells and dendritic cells. In mice, TLR9 is expressed not only in PDCs and B cells, but also in monocytes and myeloid dendritic cells derived from myeloid stem cells [5]. CpG-DNA needs to be immunized into the cell to play its stimulating effect,through the endocytosis pathway into the cell CpG-DNA is recognized and combined TLR9, activated TLR9 activation of the signal transduction.

The higher animal immune system uses TLR9 to detect unmethylated CpG-DNA in the pathogen without identifying its own DNA [6]. TLR9 is a type I membrane protein of the TLR family and is expressed in immune cells such as B cells and dendritic cells (DCs). TLR9 consists of an extracellular domain, a transmembrane region, and an intra--cellular domain. The extracellular

domain has a leucine-rich repeat sequence and a cysteine-rich domain, which is a direct effect of CpG-DNA and TLR9 Site. The extracellular area has a Toll/IL-1R homologous domain, which is necessary for TLR9 to reposition and activate later responses in the cells.

The recognition of CpG-DNA by TLR9 is specific for CpG, and the inverted or methylated CpG sequence will lose its activation to TLR9. TLR9 mediates CpG-activated DC cells, B cells, macrophages, and induces cell secretion of inflammatory cytokines and Th1-type cytokines, thereby activating natural and adaptive immune responses.TLR9 acts as an unmethylated CpG-DNA-specific recogni--tion receptor and has structural similarity to other TLR9 family receptors. TLR9 receptor extracellular domain consists of 25 lysine-rich domain and one cysteine-rich domain, the intracellular domain consists of α-helix and β-fold, the extracellular domain through the transmembrane structure Domains are linked to intracellular domains. The extracellular domain recognizes un--methylated CpG-DNA, and the intracellular domain activates the relevant protein to activate the downstream cascade signaling pathway, resulting in a corresponding immune response,such as the production of cytokines, induction of cell proliferation or apoptosis[7-8]. It was found that LRR2, LRR5 and LRR11 had strong affinity for CpG motifs in LRR, especially LRR11[9],in which LRR2 and LRR11 in LRR were activated by LRR and CpG-DNA in the extracellular domain, The At the same time, it was found that the C-terminal and N-terminal of the TLR9 extracellular domain play an important role in the activation of the TLR9 receptor, and further studies have found that the C-terminal histidine His505, glutamine Gln510, histidine His530 and serine Tyr554 TLR receptor activation is essential[10].

3.The effect of three CpG-DNA on the immune system

The immunostimulatory activity of CpG-DNA is affected by its length, the number of CpG motifs and the sequence of CpG motifs. The core CpG motif consists of hexamers with central unmethylated CpG in the usual form RRCGYY (where R represents purine and Y represents pyrimidine)[11] . At present, the researchers mainly uses artificial unmethylated CpG-ODN instead of microbial unmethylated CpG DNA for the study of immune system response to CpG-DNA. The results suggest that the CpG-DNA-TLR9 complex formed in the intracellular end may be the key to stimulate the production of IFN-α.

CpG-DNA bound TLR9 forms a TLR9-CpG-DNA dimer at a 2: 2 ratio. CpG-DNA is recognized by two protomers in the dimer, in particular the amino-terminal fragment (LRRNT--LRR10) from an protomers and the carboxy-terminal fragment (LRR20--LRR22) from another protomers[12].

However, when type A CpG-ODN was localized by ion channels and

common DC cells, it was found that these cells produced IFN-α by the MyD88-IRF7 pathway. Type B CGG-ODN can only stimulate the production of low levels of type I interferon, but can stimulate the production of high levels of inflammatory cytokines. If it is allowed to exist in the DC for a long time, can also induce the production of IFN-a[13]. These results suggest that CpG-DNA-TLR9 complex retained in the body can cause a large number of IFN-a expression.

CpG-DNA up-regulates the expression of TLR9 mRNA in activated B cells and up-regulates some of the cytokine mRNA expression that promotes Th1-type immune responses. TLR9-activated B cells and PDC expression co-stimulatory molecules CD86, CD80 and CD40 increased, promote Th1-type immune response to the secretion of chemokines and cytokines also increased, such as monocyte inflammatory protein-1 monocyte inflammatory protein-1 , MIP1, IL-6, IL-12,IP-10, etc [14] .

Dendritic cells (DCs) are important immune cells that can process and present antigens, activate T cells, and induce specific immune responses. CpG-DNA can promote the maturation of DC cells, up-regulate the expression of MHC class II molecules and costimulatory molecules, enhance the ability of DC anti-apoptosis, and secrete a variety of cytokines and inflammatory factors, including IFN-α, IL-12, IL- 6 and so on [15].After stimulation with CpG-DNA, human PDC is the most important cell producing type I IFN (IFN-α, IFN-β)[16]. In addition, the expression of DC chemokine receptors is also upregulated by CpG-DNA, which promotes the migration of PDC to the marginal and extra-T cells of lymph nodes and further promotes the acquired immune response of Th1 type [17]. CXCL14 is a native CXC chemokine that induces immature dendritic cells (DCs), tissue-resident macrophages and natural killer cell migration. CXCL14, as a vector, specifically binds to CpG-DNA and activates Toll-like receptor 9 (TLR9) to induce inflammatory cytokines such as IL-6, IL-12 p40 and TNFa in mouse bone marrow-derived dendritic cells Enhanced intracellular transport of low concentrations of CpG DNA [18].

CpG-DNA can act directly on B cells, promote its proliferation, and can activate the initial B cells, germinal center B cells and memory B cells, so that the sensitivity of the antigen to stimulate greatly increased,accelerate the differentiation of antibodies can be secreted cell. B-type CpG-ODN has the strongest immuno--stimulatory effect on B cells. Activated B cells secrete cytokines such as IL-6 and IL-10, and further stimulate the synthesis of IgM under the action of IL-6. In addition, CpG-ODN can also promote antigen presentation by up-regulating the expression of MHC class II molecules and costimulatory molecules on B cell surface [19].

NK cells are associated with T and B cells in the third group of lymphocytes,

but the number of cells is small. NK cells can directly kill the target cells, without antigen pre-sensitization, without antibody participation, and not limited by MHC. CpG-DNA can directly or indirectly promote the function of NK cells. CpG-DNA enhances NK cell activity by promoting PDC secretion of IFN-α. Tross et al.[20] found that CpG-DNA can also kill NK cells by promoting PDC secretion of chemokine ligand 5 (CCL5), thus effectively play a killing effect.

4. Regulation of CpG-DNA on Immune Signaling Pathways

CpG-DNA is an agonist of TLR9, and its immunomodulatory effect is thought to be achieved by direct or indirect activation of TLR9-expressing cells. TLR9 is distributed on the endoplasmic reticulum of the cell and recognizes unmethylated CpG dinucleotides, which can effectively identify invasive pathogen nucleic acids. Immunocytes expressing TLR9 include PDC, macrophages, B lymphocytes and monocytes [21-22]. Different types of TLR9 cell lines were different, and TLR9 expression in rodent animals was much higher than that in mammals [23]. In humans, TLR9 is mainly expressed in B cells and Plasmacytoid dendritic cells, and studies have shown that TLR9 is also expressed in activated human neutrophils, helper T cells and pulmonary epithelial cells [24-25], but its biological significance

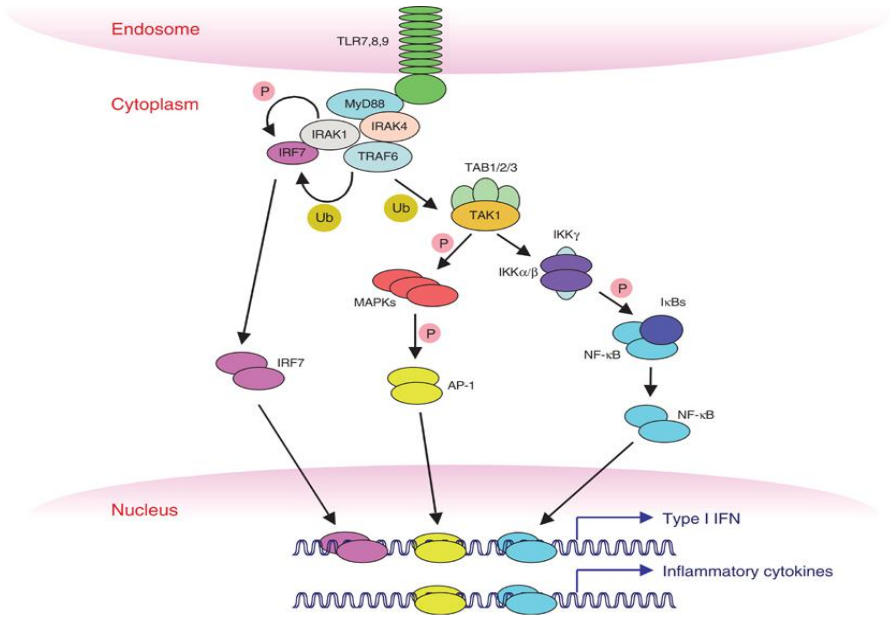

Figure: TLR3- and TLR4-mediated signaling pathways.

(Kawai T, Akira S. *Cell death and differentiation*, 2006)

is still uncertain. In mice, TLR9 is expressed not only in Plasmacytoid dendritic cells and B cells, but also in monocytes and myeloid DCs derived from myeloid stem cells [26].

MyD88 is a 35kDa cytoplasmic protein that is a common feature in the TLR9 signaling pathway and is also an important member of the TLR/IL-1R superfamily. Its C-terminal contains the TLR domain, which binds to the TIR of the intracellular domain of TLRs, and the N-terminal is the death domain. TLR9 binds to MyD88 by recruiting MyD88 to activate the down--stream signaling pathway, where IRAK4 is activated as an indispensable key molecule dependent on MyD88 [27]. Once IRAK4 is phosphorylated, it will be separated from MyD88 molecules, and then TRAF6 (TRAFs family (NF-κB) signaling pathway and the Mitogen-activated protein kinase (MAPK) signaling pathway [28]. Activated MAPK signaling pathways include p38MAPK signaling pathway, extracellular regulated protein kinases (ERK1/2) signaling pathway, and c-Jun N-terminal kinase signaling pathway. Activated signaling molecules regulate the transcrip--tion of DNA by entering the nucleus and induce the expression of various inflamma--tory cytokines and chemokines [8, 29].

In addition, the TLR9 signaling pathway also activates the transcription factor IRF7 to induce the production of type I interferons. MyD88 can form a complex with IRAK1, TRAF6, TRAF3, IKK-α and IRF7/IRF1, activating transcription factor IRF7/IRF1 into nucleus to induce type I interferon production[30]. Studies have shown that CpG DNA induces the phosphorylation of IRF7 in the presence of TRAF6 and TGF-β1 inhibits IRF7 phosphorylation, so TGF-β is suggested to inhibit CpG-DNA-induced type I IFN production by ubiquitination of TRAF6 [31].

Therefore, unmethylated CpG-DNA and cell TLR9 recognition, through the NF-κB and MAPK signaling pathway to enhance the immune response function of cells. CpG-DNA can up-regulate the expression of TLR9 mRNA in activated B cells and up-regulate the mRNA expression of cytokines that promote Th1--type immune response. The expression of CD86, CD80 and CD40 in B cells and PDCs after TLR9 activation increased Th1 Type immune response and chemokine secretion also increased, such as monocyte inflammatory protein-1, IL-6, IL-12, IP-10 and so on. The overall effect of TLR9 activation is to induce innate immunity and acquired immune systems to produce cellular and humoral immune responses that are biased towards Th1 type [32-34].

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