Global Journal of Immunology
2020; 1(1): 1-7.
ISSN Online: 0000-0000; ISSN Print: 0000-0000
Website: http://natureschol ars.com
Email: Glo_J_Imm@126.com
Publisher: Scholars Publishing, LLC

Review

The Research Progress of Nanoimmunotherapy to Treat Ischemic Heart Disease

Yao Liu [1,2,*,✉], Jun-Li Jin [3,*]

[1]Department of Clinical Medicine, The First Clinical Medical College, Yangtze University, Jingzhou, Hubei 434023, China; [2]School of Basic Medicine, Health Science Center, Yangtze University, Jingzhou, Hubei 434023, China; [3]Department of cardiovascular medicine, The First people Hospital, Jingzhou, Hubei 434023 , China;.

*** These authors contributed equally.**
✉ **Correspondence**
Yao Liu, Department of Clinical Medicine, the First Clinical Medical College, Yangtze University, Jingzhou, Hubei 434023, China. Email: 2993683095@qq.com. Telephone number: 18971124806.
Received: May 2, 2020; Accepted: September 3, 2020; Published online: October 15, 2020.
Cite this paper: Yao Liu, Jun-Li Jin. (2020) The research progress of nanoimmunotherapy to treat ischemic heart disease. *Global Journal of Immunology*, 1(1):9-16. http://natureschol ars.com/gji.010103. https://doi.org/10.46633/gji.010103.

Abstract

The mortality of cardiovascular diseases (CVDs) is gradually increasing, and atherosclerosis is the main potential cause of myocardial infarction and stroke. Atherosclerosis is resulted from cholesterol accumulation and later inflammation in the vessel wall. Although lipid-lowering therapy is still the cornerstone of atherosclerosis disease management at present, researchers have paid attention to inflammatory factors in atherosclerotic diseases as therapeutic targets. In 2017, the CANTOS trial demonstrated for the first time that the targeted IL-1β antibody canakinumab can reduce the major cardiovascular adverse events (MACE) of patients after myocardial infarction by 15%, which proved the beneficial effect of targeted anti-inflammation therapy in treating cardiovascular diseases for the first time. At the same time, animal model tests mediated by nano-immunotherapy have proved that nano-immunotherapy could reduce inflammation and prevent the progression of plaque, thus supporting the transformability of the method and the potential for the treatment of atherosclerosis.

Key words: Atherosclerosis, Ischemic Heart Disease Nano-Immunotherapy, CD40-Tumor Necrosis Factor Receptor-Related Factor 6 (TRAF6).

Introduction

Atherosclerotic cardiovascular diseases are still the main causes of vascular diseases in the world (1). Acute rupture or erosion of atherosclerotic plaque, platelet activation, secondary coronary artery thrombotic obstruction, lead to myocardial ischemia, injury or necrosis. Ruptured atherosclerotic plaque usually has a large lipid core covered by a thin fibrous cap (< 60 microns). Superficial plaque erosion is another mechanism of coronary artery thrombosis, which is different from plaque rupture. Eroded plaque is often rich in matrix, lack of lipid and macrophage colony, which is characterized by thin fibrous cap, large lipid pool and abundant foam cells(1). Binding of CD40 receptor on monocytes and macrophages to CD40 ligand on T cells is a well-known driver of atherosclerosis. Binding of CD40 receptor on monocytes and macrophages to CD40 ligand on T cells are a well-known driver of atherosclerosis. When activated, CD40 recruits tumor necrosis factor receptor-related factor 6 (TRAF6), resulting in a cascade of pro-inflammatory signal. Now researchers indicate that inhibiting CD40-TRAF6 signaling in macrophages can reduce atherosclerosis, and nano-immunotherapy that delivers TRAF6 inhibitors is safe in both mice and non-human primates(2). Furthermore, the amplification of nano-immunotherapy from mice to rabbits and pig atherosclerosis models proves that the reduction of nano-immunotherapy prevents plaque progression and supports the translatability of the method and the potential for acute treatment of atherosclerosis (3).

1. From lipid lowering to treating inflammation

Lipid-lowering therapy is still the cornerstone of atherosclerosis disease management. Evidence from epidemiological, genetic and Mendel randomized studies and randomized clinical trials involving more than 2 million participants and over 20 million annual follow-up shows that low density lipoprotein cholesterol is a causal risk factor. Therefore, early control of low density lipoprotein cholesterol is very important (4). However, with the development of cardiovascular immunopathology, the inflammatory factors of atherosclerotic diseases as a therapeutic target have attracted attention. The formation of atherosclerotic plaque is related to the recruitment of macrophage, mostly triggered by recruitment of monocyte, thus leading to atherosclerotic thrombotic events (5). The classical viewpoint is that plaque macrophages originate from circulating monocytes, which permeate the vascular wall through the process of endothelial cell regulation (6). Circulating monocytes are derived from hematopoietic stem cells in bone marrow and spleen monocyte bank. Acute stress such as myocardial infarction, mobilizes bone marrow production and spleen monocytes reservoir. This process activates monocyte recruitment to the vascular wall and exacerbates plaque inflammation (7). Research on inflammatory experimental models shows that tissue macrophages can also self-maintain through local proliferation. Experimental studies have shown that the development of atherosclerosis can be managed by reducing inflammation (6). However, whether reducing cardiovascular inflammation will translate into cardiovascular benefits for human beings has not been confirmed until 2017. CANTOS experiment challenges the mode of focusing on lipid-lowering therapy for atherosclerosis. The experiment involved 10, 061 patients, who previously diagnosed as myocardial infarction and a high-sensitivity C-reactive protein level of 2 mg or more per liter, the patients received a randomized double-blind trial that treated with placebo and canakinumab ,monoclonal antibody of interleukin -1β which is the core cytokine of inflammatory response and drives the interleukin-6 signal pathway. Canakinumab is a human monoclonal antibody against interleukin-1β, which has anti-inflammatory effect and has been approved for clinical application of rheumatism. After 3.7 years follow-up on average, Canakinumab reduced the risk of the composite end point of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death, compared with the placebo

group(8). This research is an important step to support the development of anti-inflammatory therapy to treat atherosclerotic cardiovascular diseases.

2. Characteristics of nanotherapeutic methods

Nanoparticles are a microscopic colloid system with particle size generally less than 1 μm and composed of nano microspheres and nano microcapsules. Nanoparticles can pass through tissue gaps, capillaries, blood-brain barrier and tissue endothelial cells, and release drugs at the cellular or subcellular level. In the past 20 years, nanotechnology approaches of targeted therapy have appeared in various fields of medicine (9). To achieve effective targeting, nanoparticles are designed to avoid uptake by innate immune cells (10). Doxil is the first nanodrug approved by the U.S. Food and Drug Administration in 1995 (11). Currently, more than 24 nanotherapeutic compositions ranging from oncology applications to infectious diseases are approved for clinical use (12). Although clinical trials to study new potential applications are still in progress, the application of nano-drugs in cardiovascular field is still very few. An example of a cardiovascular nano-drug is the use of liposomes containing prednisone in studies in patients with atherosclerotic diseases(13). Monocytes and macrophages as the key cells in the inflammatory response should be the primary target treat cardiovascular disease. The design and selection of specific nanomaterials should consider cell location, cell type, cell function in the environment and its role in pathological process. Not only should we consider the size, shape, drug encapsulation efficiency and in-vivo stability in nano-medical engineering, but also the toxicity of immunomodulatory drugs, especially toxicity related to liver uptake (14).

3. A Preclinical Study: TRAF6 Targeted Nano-immunotherapy for Atherosclerosis

CD40/CD40L is a pair of complementary transmembrane glycoproteins, belonging to tumor necrosis factor and tumor necrosis factor-associated activation protein, respectively. CD40/CD40L is expressed in a variety of immune and non-immune cells. CD40 is mainly expressed in B cells, antigen presenting cells and tumor cells, and CD40L is mainly expressed in activated CD+T cells and platelets. CD40/CD40L plays an important role in the occurrence, development and plaque rupture of atherosclerosis. Marnix Lameijer and his colleagues recombined high-density lipoprotein and TRAF6i-HDL, a small-molecule inhibitor of the CD40-TRAF6 interaction(15). TRAF6i-HDL is constructed from human apolipoprotein A-1 (ApoA-I) and phosphor-lipids 1- myristoyl-2- hydroxy -sn-glycerol-phosphocholine (MHPC) and 1,2-dimyristoyl-sn-glycerol -3- phosphatidylcholine (DMPC), in which a lipophilic small molecule inhibitor (SMI 6877002) that interacts with CD40-TRAF6 is encapsulated. SMI 6877002 binds to TRAF6 binding site on CD40 but does not bind to TRAF6 binding site on interleukin -1R related kinase (IRAKs), so that the signal of interleukin -1 receptor (IL-1)-Toll-like receptor is not affected. The therapy regulates the signaling of CD40-CD40 ligand in monocytes and macrophages by blocking the interaction between CD40 and TRAF6 in Apoe$^{-/-}$ atherosclerosis mouse model (15).

In vivo effect of TRAF6i-HDL on plaque inflammation. Marnix Lameijer and his colleagues used Apoe$^{-/-}$ mice of 20 weeks old, which have developed atherosclerotic lesions by feeding a high-cholesterol diet for 12 weeks .The administration of mice was intravenous infusions of either control PBS (phosphate buffer), control empty recombinant high-density-lipoprotein nanoparticles or TRAF6i- high-density lipoprotein in 7 days and then were euthanized 24 hours after the last infusion. After finishing quantitative histological analysis of plaque in aortic sinus region of mice, they found no significant difference between plaque size and collagen content in each group. However, the lesions in mice that received the TRAF6i-HDL had a

significant reduction in macrophage content by flow cytometry analysis of the whole aorta. Compared with the control group and the high density lipoprotein group, the content of aortic macrophages in the TRAF6i-high density lipoprotein treatment group was significantly reduced by 66% and 67%, respectively. Moreover, treatment with TRAF6i-HDL was associated with a significant reduction in aortic T lymphocytes content. Compared with control group and empty recombinant HDL nanoparticles group, it decreased by 65% and 49% that indicating TRAF6i-HDL has an effective anti-

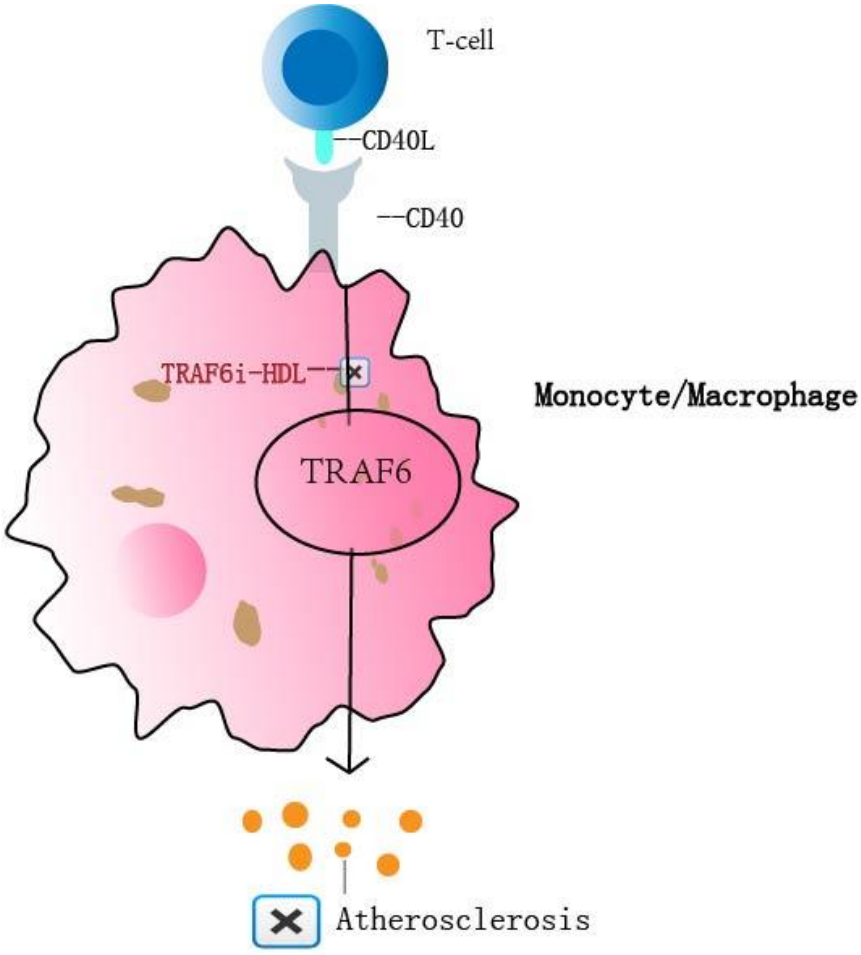

Figure 1. The combination of cd40 and cd40l leads to the recruitment of tumor necrosis factor receptor-related factors and the transmission of signals. CD40-TRAF6 interaction is the main signal transduction pathway in macrophages and a key step in atherosclerosis. Recombining the natural HDL and a TNF receptor-associated factor 6 inhibitor inhibited the CD40-TRAF6 interaction, leading to diminished monocyte recruitment.

inflammatory effect on atherosclerotic plaque after 1 week of treatment (16).

The mechanism of TRAF6i-HDL in plaque inflammation. CD68+ cells were isolated from aortic sinus plaques of the mice by laser capture microdissection. All RNA of these cells was isolated and sequenced. 416 differential genes between control group and active nanoparticles were identified, including 209 down-regulated genes and 207 up-regulated genes. Among the 15 enriched gene ontology terms that

significantly enrich differentially expressed genes, "focal adhesion" is the most concerned. Focal adhesion is a dynamic process in which protein complexes are linked to extracellular matrix and play an important role in the migration of monocytes and macrophages. The differentially expressed genes in focal adhesion are Rhoa, Rap1a and Rap1b, which play a central role in regulating monocyte migration by activating integrins. These genes are obviously down-regulated. Stable protein 1 (encoding stable protein 1) is one of the differentially expressed genes that are up-regulated; This gene is related to the phenotype of macrophages protected by atherosclerosis and is known to have lymphocyte homing and cell adhesion functions.

Table 1. Ischemic heart disease treatment.

	Characteristics	**Advantages**	**Disadvantages**
Classical lipid-lowering therapy	The observation that patients with familial hypercholesterolaemia achieve this cumulative LDL-C burden threshold at early ages and develop premature atherosclerotic CVD supports a causal role of LDL in atherosclerosis. Classical lipid-lowering therapy mostly focus on lowering LDL-C, preventing further stenosis of coronary artery.	Lipid-lowering therapy is still the cornerstone of atherosclerosis disease management. There is much evidence to prove its safety	The compliance rate of lipid-lowering is greatly affected by people's lifestyle. Furthermore, the European guidelines recommend taking the medicine for a long time. Some people could't obey well.
Anti-inflammatory therapy	Macrophage accumulation in atherosclerosis is directly linked to the destabilization and rupture of plaque, causing acute atherothrombotic events. Anti-inflammatory therapy mainly aimed at decrease plaque inflammation, reducing the risk of plaque rupture and thrombosis.	The trail discussed in the text have demonstrated that TRAF6i-HDL could reduce plaque inflammation on such a short time scale. Patients with acute coronary syndrome may be a suitable group for such inflammatory induction therapy	The clinical translation of such nano-immunotherapies and their application to treat patients with ischemic disease are challenges that lie ahead.

Discussion

CD40-CD40L signal channel has long been considered as a key role in immune response of atherosclerosis (17-20). However, therapeutic targeting of this costimulatory receptor-ligand is troublesome. Anti-CD40L antibody can effectively reduce the development of atherosclerosis in mice, but thromboembolic complications caused by platelet CD40 expression prevent its application in human beings (21). In addition, CD40 is expressed on B lymphocytes. Long-term blocking will damage its maturation and lead to immune deficiency (22). Marnix Lameijer and his colleagues solved these problems by targeting the interaction between TRAF6 and CD40 cytoplasmic domains in monocytes and macrophages. The obtained data show nano immunotherapy based on high-density lipoprotein exposes more than 80% of monocytes and macrophages to nano-targeting substances, while lymphocytes do not absorb any nanoparticles. These observations confirm previous research work on targeting efficiency of high density lipoprotein drug delivery.

Comparing with the prolonged treatment in previous therapeutic studies of targeting the CD40–CD40L signaling axis, this study minimized drug exposure through short-term treatment (1 week). It was found that the content of $Ly6C^{hi}$ monocytes and macrophages in plaque decreased by 49% and 66% respectively in one week, thus indicating the high efficiency of active nanoparticles. It is worth noting that this experiment proves that ApoA-I has only a small contribution to the therapeutic effect of TRAF6i-HDL. They used 4 infusions of 9 mg/kg ApoA-I, which was 23 times lower than the previous published studies and found no effect of high density lipoprotein on plaque monocyte or macrophage content (23).

The mechanism of TRAF6i-HDL reducing plaque inflammation on such a short timescale can be partly explained by the reduction of monocyte recruitment. In general, plaque macrophage content is determined by a balance of monocyte recruitment and macrophage proliferation, apoptosis and migratory egress. The recruitment of monocyte recruitment and macrophage proliferation are the most important factors. The experimental data did not reveal the effect on macrophage proliferation, apoptosis or migration export. However, it was observed that the content of $Ly6C^{hi}$ monocytes in plaque decreased, while the count of blood monocytes was not affected, indicating that monocyte recruitment decreased. This result is consistent with the previous observation in CD40-TRAF6 signal deficient knockout mouse model. In previous studies, CD40-T6/ mice were used to study the migration capacity of monocytes and macrophages (24).

Besides the therapeutic effects on monocytes and macrophages, TRAF6i-HDL may also have effects on endothelial cells. Adhesion molecules expressed by activated endothelial cells interact with glycosylated ligands and integrins expressed by monocytes. The experiment showed TRAF6i-HDL inhibited the migration of monocytes through endothelium *in vitro*. This result indicates that the beneficial effect of TRAF6i-HDL on endothelial cells may be attributed to the decrease of monocyte recruitment.

Until recently, no treatment specifically aimed at reducing vascular inflammation has been evaluated. As we all know, statins not only reduce cholesterol, but also have beneficial cardiovascular effects. CANTOS clinical trial provides clinical evidence to apply immunotherapy to cardiovascular diseases. However, patients who treated by Canakinumab are prone to development of serious infection. Marnix Lameijer and his colleagues designed nano-immunotherapy to rapidly inhibit plaque inflammation in patients at high risk of cardiovascular events. Targeted administration improves the curative effect while its short-term application minimizes the risks associated with long-term immunosuppression. Patients with acute

coronary syndrome may be a suitable group for such inflammatory induction therapy. Recent studies have shown that it is the initial myocardial infarction itself that causes monocytes to accumulate on atherosclerotic plaques, leading to their inflammatory attacks and prone to plaque rupture (25). Perhaps, this nano-immunotherapy is expected to be used to treat ischemic heart disease.

Summary

In the past ten years, the research on nano-immunotherapy for atherosclerosis and ischemic heart disease has been strengthened .The development of nano-engineering and cardiovascular immunology and new discoveries have also promoted the development of this research. With the safety assessment in animal experimental models and the potential for clinical transformation of nano-immunotherapy, the studies described in this review emphasize the success of this therapy. In fact, regulating immune cell response and destroying the first line of defense against pathogens is a double-edged sword. High precision is needed to select and regulate immune response, otherwise large therapeutic effects may inhibit host defense mechanisms. Nano-immunotherapy is expected to achieve the goal of highly specific targeted pathogenic processes without damaging important immune functions. The nanotechnology excellence program and the development of the seventh nanoimmunotherapy obtained from cardiopulmonary blood research are expected to change the face of cardiovascular medicine in the future.

Declarations

1) ***Consent to publication***
We declare that all authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement and followed publication ethics.

2) ***Ethical approval and consent to participants***
Not applicable.

3) ***Disclosure of conflict of interests***
We declare that no conflict of interest exists.

4) ***Funding***
None

5) ***Availability of data and material***
We declare that the data supporting the results reported in the article are available in the published article.

6) ***Authors' Contributions***
Authors contributed to this paper with the design (YL), literature search (YL), drafting (YL), revision (YL and JLJ), editing (SL and JLJ) and final approval (YL).

7) ***Acknowledgement***
None

8) ***Authors' biography***
None

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