Progress of animal experimental research on the effect of stem cells on coronary artery lesion in Kawasaki disease *

Abstract: Kawasaki disease (KD) is an acute, febrile, erupting, self-limited autoimmune disease with systemic vasculitis as the main lesion. It is more common in children under 5 years old, and the main lesions involve the coronary arteries. With the increasing incidence, it has become the first cause of acquired heart disease in children. There are many basic and clinical studies on Kawasaki disease at home and abroad, but there are few animal experimental studies on the treatment of coronary artery injury with stem cells. Therefore, it is of great value to select scientific experimental animal models for Kawasaki disease research to simulate the human characteristics of Kawasaki disease. Through the establishment and in-depth study of animal models of Kawasaki disease, it is helpful to better understand the pathophysiology, cytokines and molecular pathways related to disease progression of Kawasaki disease. At the same time, it will broaden the way for the development of new and effective diagnosis and treatment of Kawasaki disease. Therefore, based on the study of coronary artery lesion animal model of Kawasaki disease with stem cells, this article mainly discusses the research and new progress of Kawasaki disease animal models, so as to provide a good theoretical basis for clinical and animal experimental research. And make a review on this issue.

Key words: Stem cells, Kawasaki disease, Coronary artery lesion, Animal model, Research, Progress

Introduction

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is an acute febrile disease and systemic vasculitis with inflammatory lesions of the small and medium-sized arteries throughout the body[1].It occurs in children under 5 years of age and has a worldwide incidence, although the incidence often varies widely by region. In addition, studies in developed countries have found that the incidence of Kawasaki disease is increasing[2]. Kawasaki disease was first discovered by a Japanese paediatrician, Tomisaku Kawasaki, and reported in the 1967 Japanese edition of the medical journal Allergy. The most prominent clinical manifestations of the disease are fever, polymorphic or scarlet fever-like rash, non-purulent bilateral

conjunctival congestion, congested and raised tongue papillae, chapped lips, swollen superficial lymph nodes in the neck, stiff swelling of the hands and feet, and membranous desquamation of the extremities and perianal area, making it a serious threat to the physical and mental health of children. Coronary artery lesions (CAL), the most serious complication of Kawasaki disease, are associated with coronary artery blockage and atherosclerosis in adulthood. Coronary artery arteritis and coronary artery aneurysm (CAA) occur in up to 30% of untreated children[3]. Although in children treated with high doses of intravenous immunoglobulin (IVIG), this percentage is reduced to 5-7%. However, the exact mechanism by which IVIG reduces the incidence of cardiovascular complications remains unclear. Up to 15-20% of patients with Kawasaki disease do not respond to IVIG treatment and the incidence of CAA is significantly increased in these patients[4].Severe cases can lead to myocardial infarction, sudden death and localised ischaemic heart disease, and some children even develop giant coronary aneurysms, which is the leading cause of acquired heart disease in children [5,6].

The cause of Kawasaki disease is unknown and the pathogenesis is not yet clear. It is generally accepted that the disease may be caused by the invasion of one or more known or unknown microorganisms into a genetically predisposed susceptible population (particularly in Asians) which activates an immune response involving multiple T and B cells, triggering a cascade of cytokines in the body that ultimately leads to widespread endothelial damage, remodelling and dysfunction throughout the body[7].The current understanding of the pathogenesis of Kawasaki disease is limited by a number of factors: the low availability of human tissues and the failure to identify the specific cause of the disease, since the main aggressive population of the disease is young children and the incomplete understanding of the molecular and cellular mechanisms that lead to cardiovascular sequelae. Therefore, experimental animal models that mimic the human features of Kawasaki disease and their translational utility are of great value to the study of Kawasaki disease. The study of animal models contributes to a better understanding of the pathophysiology of Kawasaki disease and the mechanisms of cytokines and molecular pathways involved in disease progression. At the same time,the evidence of effectiveness we obtain from the animal models of Kawasaki disease could pave the way for the development of new and effective therapies for Kawasaki disease.

Therefore, current animal models are also based on the intersection of the infection and immune theories, which suggest that bacterial superantigens are an important cause of Kawasaki disease and that the abnormal immune activation of Kawasaki disease is mediated by bacterial or viral toxins by a superantigenic mechanism. The main modelling factors used today in animal models of Kawasaki disease are based on the superantigen theory. The modelling factors used today are immune-inducing agents such as[4], candida albieans-derived substances (CADS), lactobacillus casei cell wall extract (LCWE), candida albieans water-soluble polysaccharide fraction (CAWS), etc. In addition, the prototype of immune vasculitis is a serum disease; therefore, some studies have applied allogeneic proteins, such as: bovine serum albumin and horse serum, as the immune inducers to make animal models of Kawasaki disease.

Based on this, there is an urgent need to construct an ideal animal model that can reasonably simulate KD as close as possible, so as to facilitate the study of the etiology and pathogenesis of KD and the development of new therapeutic targets, but up to now, the best model for basic and clinical research of KD has not been found in the world. In this paper, we review the animal models of Kawasaki disease that have been established at home and abroad.

1. Mouse model

The mouse is the most commonly used animal for Kawasaki disease models, with a wide range of strains, easy access to materials and lower costs than other animals. For different experimental purposes, a number of immunocompetent mice and pure-line mice with different genetic defects were selected for control, such as BALB/C mice, CBA/N mice, nude A/J and nude C57BL/6 mice, C57BL/6-bg grey mice, etc., to study the role of T cells, B cells, monocytes, various cytokines and complement in the pathogenesis of KD, thus providing a good help to explore the key aspects of the pathogenesis. Commonly used mouse strains include C57BL/6J, C3H/HeN, CDI, DBA/2N and Ba1b/c,etc. Among them, C57BL/6J and C3H/HeN mice are highly susceptible to LCWE-induced Kawasaki disease, and most of them use wild female mice aged 4-5 weeks[8].The specific immune-inducing agents involved were Candida albieans-derived substances (CADS), Candida albicans water-soluble fraction (CAWS) , Lactobacillus casei cell wall extract (LCWE).

We therefore investigated the histological features of coronary artery (CA) stenosis in mice induced by Lactobacillus casei cell wall extract (LCWE). LCWE-induced coronary inflammation gradually progressed in a time-dependent manner and expanded to all layers of the vessel wall over 28 days[9]. In addition, frequent elastin degradation was observed and abundant α-smooth muscle actin (SMA)-positive vascular smooth muscle cells (VSMCs) infiltrated into the intima. Furthermore, most VSMCs were positive for proliferating cell nuclear antigen (PCNA) following staining, suggesting that VSMCs likely exhibited a proliferative phenotype. In conclusion, we show a novel mouse model of coronary stenosis induced by LCWE that is charact[10].

Candida albicans is a harmless commensal fungus usually found in the human gastrointestinal tract that can transform into a pathogen capable of inducing inflammation in an immune compromised host. It is usually found in the oral cavity, upper respiratory tract, intestine and vagina of normal humans, and its cell wall components contain mannoproteins, β-1,6-glucans, and β-1,3-glucans proteins[11,12]. Hisao Murata et al. alused the alkaline extracts of Candida albicans cell wall (CADS) isolated from the faeces of KD patients to induce coronary arteritis in mice, and found that the vascular pathology was similar to the coronary artery injury in KD, and successfully replicated this mouse model [13,14].Subsequent studies have found that different strains of mice had different responses to CADS[15].The incidence of arteritis in CD-1 mice is 66%, with the most frequent sites of involvement being the epicoronary coronary arteries and the aortic roots emanating from the coronary arteries. The incidence of coronary arteritis in mice of the C3H/HeN, C57BL/6N and DBA/2N strains was 100%, with the most severe inflammation in mice of the DBA/2N strain,while the incidence of coronary arteritis was only 10% in the CAB/JN strain of mice. The histological features of the Kawasaki disease model induced by this method were most similar to those of human Kawasaki disease. Ishida-Okawara et al.[16] found that in the wild-type 57BL/6 mouse model, inflammatory reactions were seen in all layers of the coronary and aorta, with a large number of neutrophils, macrophages and a small number of lymphocytes infiltrating,the outer elastic layer of the vessel is disrupted. Focal fibrinoid necrosis and varying degrees of fibrosis could be seen in the outer membrane, while arteritis or small vessel vasculitis was almost absent in the liver, kidney and lung.

Candida albicans water-soluble fraction (CAWS), which contains major cell wall components such as mannoproteins and β-glucan, and is completely decontaminated with endotoxin, peptidoglycan and nucleic acids, has various biological effects such as inducing leukocyte and platelet proliferation, altering vascular permeability, causing acute shock and CAL in vivo. K. Takahashi et al.[15,17] used a similar method to isolate Candida albicans from the faeces of patients with KD and extracted its cell wall components, which were then injected intraperitoneally five times in mice to induce coronary arteritis. The incidence of arteritis was found to be 66%, with the coronary arteries and aortic roots being the most frequently involved. The main pathological features are inflammatory infiltration of the vessels, marked thickening of the fibroblastic intima with the inner and middle elastic membrane disruption, and coronary dilation, suggesting that the pathological features have many similarities to those of KD coronary injury. The inflammation can also affect non-coronary sites in mice and can be observed in the lymph nodes, kidneys and liver.

Lactobacillus casei is a gram-positive bacterium that colonises the gastrointestinal and urogenital tracts of both humans and animals. LCWE is an extract of the cell wall of Lactobacillus casei. The peptidoglycan of this cell wall is L-D-aspartate, and in addition, the cell wall contains aminogalactose, rhamnose, galactose, etc. The composition of its special antigenic determinant cluster is similar to that of some components of the cell wall of Streptococcus A. It shares antigenic properties with certain glycoproteins in human blood vessels, myocardium and other tissues ,and it is therefore hypothesized that LCWE may induce an autoimmune response in the body under certain conditions through molecular mimetic mechanisms, leading to immune damage to blood vessels, myocardium and other tissues. Studies in animal models have found that in addition to coronary arteritis, LCWE can also cause damage to some organs outside the cardiovascular system, such as lung, kidney, liver and so on[18].Lehman et al.[19] were the first to use ultrasonic fragmentation to extract LCWE and inject it intraperitoneally into LEW/N female rats to induce polyarthritis in 1983. They induced coronary arteritis in C57BL/6 mice by a single intraperitoneal injection of group B lactobacillus casei cell wall components[20].The specific method was: C57BL/6J mice injected abdominally and intramuscularly with 0.1ml of LCWE (containing 200mg LCWE). On days 1, 3 and 28, the white blood cell count gradually increased, and platelets and mean platelet volume (MPV)

increased on day 3 after LCWE injection, and basically returned to normal on day 28; the pattern of change was similar to that of PLT in clinical patients with Kawasaki disease.Pathological sections of the heart of LCWE mice showed thickening of the blood vessel wall and infiltration of inflammatory cells around it, some of which may appear as plaques.The LCWE-induced cardiovascular lesions in mice are histologically similar to those observed in human disease.The LCWE-induced vasculitis in Kawasaki disease is characterised by inflammatory cell infiltration of the aortic root and necrotizing arteritis of the coronary arteries, followed by luminal obstruction due to LMP, leading to complete coronary artery stenosis[21].

It was found that the cell wall of Lactobacillus casei has superantigenic activity and does not require antigen-presenting cell processing prior to recognition with T cells, and that when LCWE is injected intraperitoneally, it generates T cell appreciation and expression of the T cell receptor Vβ, triggering a superantigenic response.The LCWE intraperitoneally injected mouse model is similar to KD in terms of coronary artery pathological changes, course of development, response to IVIG, and superantigen etiology, and is a more mature animal model of KD coronary artery coronary lesions[22,23].In 2007, Nakamura et al. first immunized C57BL/6 mice with Bacille Calmette-Guérin vaccine (BCG) made from atypical mycobacteria and induced autoimmune vasculitis[24] .

In summary, Kawasaki disease vasculitis can be induced in mice by injection of casein, cell wall components of Candida albicans, etc. These mouse models of Kawasaki disease have accelerated the study and understanding of the pathogenesis of Kawasaki disease in humans. However, no animal model can perfectly reproduce human disease. Especially in the context of the unclear etiology of Kawasaki disease, researchers must be cautious when interpreting the results based on experimental models and confirming findings in patient cohorts. We cannot simply extrapolate preclinical mouse data to humans, but mouse models remain valuable tools for studying certain aspects of the pathogenesis of human disease.

2. Young rabbit model

In1995, Onouchi Z[25] injected horse serum into the veins of newly weaned young rabbits for 2 weeks and induced coronary artery dilatation and coronary aneurysm formation of young rabbits. In contrast, adult rabbits could develop similar

coronary arteritis and myocarditis , but there were no dilated coronary artery changes. This reveals the important fact that type II hypersensitivity reactions due to serum sickness or intravenous administration of allogeneic serum proteins can lead to coronary artery dilation in rabbits. However, this modelling approach of using an allogeneic serum protein component to induce a type II hypersensitivity reaction does not fit well with the fact that the cause of KD is unclear. In China, Wei Weizhong[26,27] et al. referred to the method of Onouchi et al. and for the first time used repeated intravenous injections of bovine serum albumin to cause immune inflammatory injury in rabbits' blood vessels instead, and performed vascular wall elastin fiber staining and coronary artery ultramicro-pathological examination. The pathological changes of immune vasculitis induced by bovine serum albumin in rabbits were found to be similar to those of KD, and only young rabbits could cause this significant coronary lesions, while adult rabbits could not. According to Weiwei et al, the tissue structure of the mammalian coronary artery is underdeveloped during juvenile life and is located at the beginning of the aorta, which is subject to the highest vascular pressure, combined with the anatomical characteristics of travelling on the surface of the heart without the support and protection of surrounding tissue. Regardless of the cause of the vasculitis, as long as the inflammatory injury involves the elastic fibre layer and the muscular layer of the vessel, causing damage to the elastic layer and muscular layer within the arterial wall, and the vessel is unable to withstand the pressure of blood flow that normal tissue can withstand, which may cause the affected coronary artery to dilate. In contrast, adult rabbits have a more histologically developed vessel wall and a different degree of immune response to exogenous antigens, resulting in mild coronary pathological damage and no changes such as coronary dilation[28].

3. Dog model

In 1991, Burns et al.[29] reported that the clinical presentation, ancillary examination and pathology of polyarteritis syndrome in puppies were similar to those of KD. In 1992, Felsburg[30] et al. similarly identified "canine pain syndrome" in dogs, known as polyarteritis in puppies, a systemic necrotizing vasculitis of unknown etiology, involving mainly small and medium-sized arteries, most likely the coronary arteries, and occurring mainly in puppies between the ages of 3 and 18 months. The main clinical manifestations are fever, gait stiffness, cervical pain and cervical

stiffness; the laboratory tests revealed a significant increase in leukocytes and neutrophils and an increase in serum IgA; a significant increase in peripheral blood B lymphocytes and a decrease in T lymphocytes; and a significant suppression of the stress response to mitogenic stimuli. Therefore, it is suggested that spontaneous polyarteritis in puppies may provide a new clue to study the etiology and pathogenesis of KD. However, there are no recent studies on this canine model and no one has replicated this model.

4. Pig model

In 2004, Philip et al.[31] repeated intramuscular injections of horse serum into small piglets and immediately after serum injection, different degrees of skin erythema were observed immediately in different parts of the piglets such as legs, chest and ears, mouth and perioral area, perineum and perianal area, and in piglets over 2.5 months of age, redness of the skin, increased heart rate and chills were observed within 30 minutes after injection. The cardiac ultrasound findings showed varying degrees of coronary artery dilatation; Pathological examination showed a variety of changes, such as inner elastic membrane disruption and endothelial hyperplasia, and skin biopsy showed perivascular inflammatory infiltration. The piglet model, injected with horse serum, showed a rash and coronary dilation similar to that of KD and a pathology similar to that of the subacute phase of KD. This model was successfully replicated by Philip et al. in recent years and is considered to be a tool for the study of the pathogenesis and treatment of immune vasculitis. The rash, ultrasound and histopathological manifestations of this model are very similar to those of human Kawasaki disease and may be a specific animal model for immune deposition vasculitis, which could be very useful for future developments in the treatment of KD. This model has not yet been replicated in piglets in China.

Over the past 40 years, scholars have observed biological data from blood specimens of children with Kawasaki disease, combined with research theories from experimental animal models of Kawasaki disease, these continued basic and clinical studies have improved our understanding of the pathogenesis of Kawasaki disease and coronary vasculitis. However, some questions remain unanswered, such as the determination of the etiology, how the disease is triggered, and the specific immune pathways associated with the development of coronary vasculitis and IVIG resistance.

As human tissue from Kawasaki disease patients is very rare, the use of animal models that replicate the characteristics of human Kawasaki disease is invaluable. We still need this "bench to bedside" approach to explore the etiology and pathogenesis of Kawasaki disease and to explore new ways of treating Kawasaki disease and reducing the incidence of CAL.

Conclusion

The establishment of animal models of Kawasaki disease provides a good basis for research on the etiology, pathogenesis, prevention and diagnosis of Kawasaki disease, but the current animal models are only partially close to the clinical manifestations or pathogenic processes of Kawasaki disease. In the future, we will use molecular and cellular biotechnology, together with the aid of small animal imaging techniques, to combine KD animal models and KD patients to develop more ideal animal models of Kawasaki disease.

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