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Review:

Advances in monoclonal antibody application in myocarditis*

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Abstract: Monoclonal antibodies have become a part of daily preparation technologies in many laboratories. Attempts have been made to apply monoclonal antibodies to open a new train of thought for clinical treatments of autoimmune diseases, inflammatory diseases, cancer, and other immune-associated diseases. This paper is a prospective review to anticipate that monoclonal antibody application in the treatment of myocarditis, an inflammatory disease of the heart, could be a novel approach in the future. In order to better understand the current state of the art in monoclonal antibody techniques and advance applications in myocarditis, we, through a significant amount of literature research both domestic and abroad, developed a systematic elaboration of monoclonal antibodies, pathogenesis of myocarditis, and application of monoclonal antibodies in myocarditis. This paper presents review of the literature of some therapeutic aspects of monoclonal antibodies in myocarditis and dilated cardiomyopathy to demonstrate the advance of monoclonal antibody application in myocarditis and a strong anticipation that monoclonal antibody application may supply an effective therapeutic approach to relieve the severity of myocarditis in the future. Under conventional therapy, myocarditis is typically associated with congestive heart failure as a progressive outcome, indicating the need for alternative therapeutic strategies to improve long-term results. Reviewing some therapeutic aspects of monoclonal antibodies in myocarditis, we recently found that monoclonal antibodies with high purity and strong specificity can accurately act on target and achieve definite progress in the treatment of viral myocarditis in rat model and may meet the need above. However, several issues remain. The technology on how to make a higher homologous and weak immunogenic humanized or human source antibody and the treatment mechanism of monoclonal antibodies may provide solutions for these open issues. If we are to further stimulate progress in the area of clinical decision support, we must continue to develop and refine our understanding and use of monoclonal antibodies in myocarditis.

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1 Introduction

In 1975, Köhler and Milstein (1975), professors of Molecular Biology at the University of Cambridge, successfully prepared anti-sheep red cell monoclonal antibodies in their laboratory, then they set up a method for preparing monoclonal antibodies by the hybridoma technique, which is using spleen cells of mice immunized with a predetermined antigen in fuse with unrestricted growth of myeloma cells in vitro to form a B cell hybridoma. It produces antibodies that are directed to the same antigenic determinants with highly homogeneous antibodies, referred to as the monoclonal antibody (McAb). Compared with polyclonal antibodies, monoclonal antibodies have a high purity, predefined specificity, and good reproducibility, and can offer a continuous supply. The advent of monoclonal antibody technology not only brings a revolution in the field of immunology in the biomedical sciences but also promotes the development of many disciplines. Meanwhile the technology is widely used in all medical areas. Monoclonal

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antibodies have become an important clinical diagnostic method and effective pharmacotherapy, and have been widely used for the treatments of autoimmune disorders, inflammatory diseases, cancer, cardiovascular disease, organ transplantation, and infectious diseases, etc.

2 Monoclonal antibody development

Since the murine monoclonal antibody came into being, monoclonal antibodies have experienced four stages of development, which are murine antibodies, rat/human chimeric antibodies, humanized antibodies, and human antibodies (Gonzales *et al.*, 2005).

2.1 Murine monoclonal antibodies

Most of current productions of McAbs are the murine type. In the clinical application, there are still many problems. The murine monoclonal antibody shows a weak affinity to crystallizable fragment (Fc) receptors on the surface of immune cells, weak killing ability, and short duration. Murine McAb also has immunogenicity, so it produces human anti-mouse antibodies (HAMAs), which reduces the effect of the monoclonal treatment, and may induce allergic reactions.

2.2 Rat/human chimeric antibodies

Chimeric antibody is produced by gene recombination techniques employing the human McAb constant region genes to replace the murine McAb constant region genes. Therefore, it codes to produce the McAb which retains its antigen binding activity and reduces murine antibody immunogenicity as much as possible. Chimeric antibodies still retained 30% murine antigen, so it can induce human anti-mouse response.

2.3 Humanized antibodies

Humanization is mainly a reconstruction of antibodies and surficial remodeling of antibodies. Reconstructed antibodies still contain residual amounts of heterologous genes, which can cause immune rejection. It also has the shortcoming of low affinity and low specificity. Surficial remodeling of antibodies is focused on rehabilitation or replacement of amino acid residues which are obviously different from human's. Based on the need to maintain antibody activity and reduce heterology, it chooses the certain amino acid residues which are similar to human's to take the place of the differential ones (Lin and Yan, 2004; Roopenian and Akilesh, 2007).

2.4 Human antibodies

Fully human antibodies are the most ideal antibodies for the treatment. Preparation methods commonly used are antibody phage antibody library technology, ribosome display technology, and genetically engineered mice methods. However, within these technologies there can also exist certain defects. For example, affinity of antibodies acquired from a non-immune antibody library is lower, immune antibody library capacity is limited, and the high affinity of low copy specific phage antibodies is lost in the screening process. Ribosome display technology for preparation of antibodies consists of large storage capacity, predefined specificity, and high affinity. Fully human antibodies prepared using genetically engineered mice are peripheral blood lymphocytes, which are from the immunized donor or cancer patients, transplanted in severe combined immunodeficiency mice, and the human antigen can be obtained after antigen immunization (Filpula, 2007).

Phage antibody library technology can rapidly separate the highly similar affinity antibodies from the real antigens. The acquired antibodies can be used in the preparation of completely humanized antibodies. Phage antibody library technology is by far the most mature and widely used antibody library technology. It also has some limitations such as storage capacity, codon bias, and amino acid modified by host restriction, and the technology is dependent on intracellular gene expression. So some antibodies are toxic to cells and the antibodies are very difficult to obtain an effective expression. The established phage antibodies have two types: immune antibody library and non-immune antibody library. So far the most mature and widely used antibody library, the phage antibody library has the following features. The biggest feature is the realization of the unity of genotype and phenotype; the phenotype of phage antibody library is a fully human antibody and it acts mainly as active fragments of single chain variable fragments

(scFv), antigen-binding fragments (Fab), and other forms. Their tissue penetrating and antigen binding have obvious advantages as compared to the intact antibodies; it can select the specific antibody genes after many applications of the "adsorption-elution-amplification" enrichment process. It has a large capacity for screening. For example, hybridoma technology screening capacity usually reaches a thousand clones, while antibody library technology can reach more than 10^6 clones. The scale of usage is extensive, and the use of phage display technology for screening antibodies has a lot of features, such as a high specificity, is easy to preserve, has a short production cycle, has an easy mass production, and so on. It shows obvious advantages in the process of mass production (Wu *et al.*, 2007). Ribosome display technology is affected by two factors: (1) the affinity of the antibody from never immunized animals is not highly acquired; (2) antibody library capacity is insufficient to cover some animal antibody diversity due to the limited exogenous gene transformation rate. Large capacity antibody library is the key to acquire high-affinity antibodies and rare special antibodies.

3 Etiology and pathogenesis of autoimmune myocarditis

Myocarditis is an inflammatory disease of the heart and a precursor of dilated cardiomyopathy (Woodruff, 1980; Maisch *et al.*, 1982; 1983; Aretz, 1987; Aretz *et al.*, 1987; Brown and O'Connell, 1995; Caforio *et al.*, 1996; Felker *et al.*, 1999). Myocarditis is often characterized by a cellular infiltrate, and if inflammation of the myocardium does not resolve at the acute stage, the heart may be compromised due to necrosis and direct loss of myocytes (Huber *et al.*, 1980), injury from granulomatous inflammation (Cooper *et al.*, 1997; Cooper, 2000), or fibrosis due to proliferation of fibroblasts and collagen deposition (Fairweather *et al.*, 2004; 2005), which can lead to dilated myocardiopathy and ultimately to congestive heart failure (Cooper, 2009). Myocarditis etiologies include infectious and non-infectious varieties. Virus infection is the most common primary example of human lymphocytic myocarditis, such as those of enteric viruses (especially Coxsackie virus B (CVB), Kirsach virus), adenovirus, herpes simplex virus, and *Trypanosoma cruzi* (Kuhl *et al.*, 2005; Rose, 2009). However, for a subset of patients with the disease, common causal infections are undetectable, but autoantibodies against cardiac antigens are present and symptoms improve following immunosuppressive treatments (Metzger and Anderson, 2011). Nevertheless, some evidence supports a transition from a virally instigated heart inflammation to activation of the immune system against self antigens. The heterogeneous nature of myocarditis in humans makes diagnosis and treatment decisions difficult (Cooper *et al.*, 2006). Monoclonal antibody application for myocarditis may bring a new horizon to pharmacotherapy.

An animal model of viral myocarditis (VMC), which is a T cell-mediated autoimmune disease and can develop into dilated cardiomyopathy, is established via CVB3, a member of the Picornaviridae family (Fairweather *et al.*, 2001; Fairweather and Rose, 2007). Studies show that in VMC mice, myocardial histological changes are similar to anthropology lymphocytic myocarditis (Yuan *et al.*, 2010b), and thus this model has been widely used for studying both the acute infectious phase and the chronic immune phase of human VMC. Both the direct viral response and immune-mediated mechanisms have been shown to contribute to the pathogenesis of acute injury and subsequent cardiac remodeling (McManus *et al.*, 1993; Cooper, 2003). In the acute period, a few sporadic small nidi of myocyte necrosis occur only in 1 to 3 d after viral infection. And a virus-specific cytotoxic T lymphocyte can react against and dissolve any uninfected cardiac myocytes. In two weeks, with the virus removed from the body, results show an increase of infiltration of the inflammatory cells and a decrease of necrotic myocardium with subsequent fibrosis and calcification in the surface by degrees on Day 7, and most animals were rehabilitated. However, the body has produced myocardial ingredient antibodies after that infection (Schulze and Schultheiss, 1995; Caforio *et al.*, 2008; Root-Bernstein *et al.*, 2009; Zhang *et al.*, 2010). Autoimmune inflammation continued without the virus for weeks and months, eventually resulting in dilated cardiomyopathy which presents itself as diffuse myocardial necrosis with multinucleated giant cell infiltration. The detection rate of cardiac muscle globulin (or other primary

autoimmune) autoantibodies in circulation for patients with autoimmune myocarditis is high (Nussinovitch and Shoenfeld, 2010; Staudt *et al.*, 2010).

Experimental autoimmune myocarditis (EAM), which was induced in a susceptible rat immunized by purified cardiac myosin, is similar to human myocarditis and can develop into DCM. It has become a good experimental animal model for study of autoimmune reaction mechanism and to explore the new measures.

Cytokine plays an important role in the development from VMC to dilated cardiomyopathy. Early antiviral responses and subsequent immune myocardial damage are mediated by cytokines. In vivo and in vitro studies show that many cytokines can suppress myocardial contractility and it is one of the main pathophysiologic mechanisms. In the murine myocarditis model, cytokines are associated with virus gene expression. New studies have shown that in a patient with acute myocarditis, circulating levels of interleukin 1α (IL-1α), IL-1β, tumor necrosis factor α (TNF-α), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF) rise (Kaya *et al.*, 2008; Shimada *et al.*, 2010).

4 Application of monoclonal antibodies in myocarditis

4.1 Monoclonal antibody therapy against a viral replication period

Eosinophilic myocardial virus leads to cell lysis through intracellular viral replication. Self-antigens (such as myosin, adenine nucleotide translocator (ANT)) released after virus-specific immune injuries, acting on the antigen-presenting cells (APC) to activate $CD4^+$ Th cells and $CD8^+$ T cells, and promote the development of B cell proliferation and differentiation. It produces double damage on myocytes through the cellular and humoral immune mechanisms (Staudt *et al.*, 2002). Amino acid sequence from 27 to 36 on ANT protein was structurally similar to amino acid sequences on 1218–1228 of CVB3 coat protein. Therefore, antibody to CVB3 can react with ANT protein molecules and take cross-reactive antigens with cross-reactive antigen of calcium influx which can cause intracellular calcium overload, which leads to myocardial injury and damage (Cunningham, 2004).

It is a well established fact that in the sub-acute stage of CVB3 myocarditis (Days 4–14), excessive immune responses become the dominant damage factor instead of virus virulence (Esfandiarei and Mcmanus, 2008). Th1-dominant immunity has been considered as one of the important mechanisms in the development of CVB3 myocarditis, and the shift of Th1 to Th2 immune response could alleviate the myocarditis severity (Frisancho-Kiss *et al.*, 2009). IP-10, a Th1-type chemokine, plays a key role in many Th1-mediated diseases. Thus, blocking the IP-10 signaling pathway may result in decreasing the induction and recruitment of Th1 cells to the local tissue site and improve organ functions (Yue *et al.*, 2011). Therefore, we can easily forecast that using an IP-10 McAb competitively binding the special receptor as a treatment to VMC after CVB3 infection may effectively ameliorate myocarditis features.

Studies in vitro found that myocardial cells stimulated by IFN-γ can induce expression of CD40 on the myocardial cell. Seko *et al.* (1998) found that myocardial cells stimulated by CD40 monoclonal antibody can produce IL-6. CD40L/B7-1 monoclonal antibody treatment in VMC rats can significantly slow down the onset and development of acute myocarditis.

4.2 Monoclonal antibody therapy against the autoimmune reaction

Liao *et al.* (2005) used L(3)T(4) McAb to treat DCM in BALC/c mice immunized by ANT carrier peptide, and the result showed that there was no DCM myocardial tissue pathological change in the treatment group and suggested that the L(3)T(4) McAb can induce ANT carrier peptide infectious tolerance and avoid the incidence of DCM in animal models.

EAM is a T cell-mediated autoimmune disease, the $CD4^+$ T cell plays a most important role in the occurrence and development of the disease, because most of the secreted cytokines are essential for activation of the immune response.

Based on studies of the model of VMC and EAM, Th1, Th2, and Th17 are found to play an important role in the process of immune responses in acute myocarditis to DCM (Fuse *et al.*, 2001; Han *et al.*,

2011b). Traditional views reported that acute VMC was due to increased immune response induced by Th1, but current studies found that Th1 type immune response was capable of inhibiting type Th2 immune response, reducing the viral replication, and preventing its further development into chronic myocarditis and DCM. The mechanism of Th2 inhibiting the Th1 immune response and acute myocarditis is done through decreasing the regulatory T cells (Treg) and inflammatory cytokines (Fuse *et al.*, 2003), but the result is harmful, because it will induce acute myocardial remodeling, and then result in chronic myocarditis and DCM. The tendency toward a Th2 immune reaction is likely to conduce to a worse disease progress of mice of the resistant strains from myocarditis progress to DCM. IL-17A, signaling molecules of Th17 cytokine, does not appear in great quantities during the acute inflammatory phase. However, IL-17A has played an important role in cardiac remodeling and the occurrence of the DCM process (Mabry *et al.*, 2010). Currently, in regards of Th1/Th2 immune balance, Th17-mediated immune reaction has become a research hotspot of myocarditis monoclonal antibodies.

Recently, some studies (Harrington *et al.*, 2005; Steinman, 2007; Korn *et al.*, 2009; Miossec, 2009; Yuan *et al.*, 2010a; 2010c) pointed out that IL-17, widely known as a Th17-derived proinflammatory cytokine, may serve as a vital function for inflammatory and autoimmunity diseases, which further supports the concept of IL-17 being targeted for treatment. Using IL-17 McAb-treated VMC mice, Fan *et al.* (2011) found that neutralizing IL-17 with anti-IL-17 can improve myocarditis manifestation and retard the diseases course. Congruously, histological analysis of heart sections presented the fact that IL-17 McAb can attenuate the inflammation and clinical sign of myocarditis, and improve pathological features. Significantly, recent clinical trials with short duration immunosuppressive therapy in established rheumatoid arthritis (RA) provide the direct evidence of the pathological role of IL-17 in RA, and indicate that a potential valid approach to treat immunologic disease by IL-17 antagonists' therapy (Genovese *et al.*, 2010).

Existing researches show that anti-CD4 monoclonal antibody in vivo can combine with CD4 molecules to intervene in the function of $CD4^+$ T cells and induce immune tolerance. Monoclonal antibody therapy toward CD4 in EAM rats can significantly reduce type Th1 cytokine concentrations and significantly regulate the type Th2 cytokine concentrations. Wang *et al.* (2006) used porcine myosin to induce EAM in rats and treated the animals with anti-CD4 monoclonal antibodies. They found significantly increased cardiac function in the treatment group compared with the untreated animals, amelioration in cardiac histopathology, and an inhibitory effect on the production of anticardiac myosin antibodies. Serum levels of Th1 cytokines were significantly down-regulated by antibody administration, while the production of Th2 cytokines was up-regulated or unaffected.

CD28 McAb can reduce the mortality of VMC mice, reduce inflammation, inhibit viral replication, increase peripheral blood levels of IFN-γ, and elevate the level of IL-4 in peripheral blood, as well as regulating the mRNA in myocardial tissue, which indicates that CD28 McAb can make the VMC mouse Th1 response decrease, the Th2 response be enhanced, and also the Th1/Th2 balance switch to a Th2 offset. Chen *et al.* (2011) showed that CTLA4-Ig and anti-CD40L monoclonal antibodies in EAM rats can decrease the inflammatory infiltration and delay myocardial injury in treatment for EAM rats, and furthermore the combination of the two drugs is better than a single one.

Inomata *et al.* (2000) showed that the application of monoclonal antibody OX34 toward CD2 prior to myosin immune injection can completely avoid the occurrence of EAM in Lewis rats. The application of monoclonal antibody OX34 after appearance of myocardial injury for EAM prevention was partially effective. After OX34 treatment, flow cytometry detection of lymph node cells showed no occurrence of proliferative response of lymphocytes under myosin stimulation. However, when combined with IL-2 stimulation, a lymphocyte proliferation reaction appeared. The results indicated that the application of CD2 monoclonal antibody OX34 prevents EAM through the consumption of T cells, and mainly through the attenuation of the Th1 function.

In addition to the above factors, other factors involved in the immune response also play an important

role in the occurrence and development of myocarditis. High mobility group protein B1 (HMGB1) is a member of the HMG protein family, which is expressed widely in a variety of tissues and cells with the function of regulation of gene transcription, stabilization of nuclei, and release of inflammatory mediators. So it is a kind of important molecular recognition mode (damage associated molecular patterns, DAMPs).

Necrotic cells passively release HMGB1, whereas activated macrophage (Mφ) and dendritic cells (DCs) actively secrete HMGB1 which is combined with its ligand receptor for advanced glycation end products (RAGE) and Toll-like receptors (TLRs) 2, 4, 7 to play a role in regulating immune response, and also is involved in tumor, ischemia reperfusion injury, and autoimmune disease development. Su *et al.* (2011), using the homemade anti HMGB1 B box monoclonal antibody, neutralized HMGB1 in circulation to effectively attenuate myocardial pathological damage and reduce Th17 cell infiltration. This prompted that HMGB1 plays a regulatory role on Thl7, due to myocardial cell injury or immune cell infiltration with releasing HMGB1 in myocarditis. HMGB1 is expected to become a potential therapeutic target for autoimmune myocarditis.

Myocardial changes in rats immunized with cardiac myosin show similar characteristics (Rose, 2009; Han *et al.*, 2011a). And the pathogenesis is similar to the second stage of VMC. Mascaro-Blanco *et al.* (2008) found a new class of cross-reactive autoantibodies against human cardiac myosin, whose epitopes can also be widely considered as the disease-specific peptide epitopes in cardiomyopathies. In addition, they pointed out that these epitopes were found primarily in the S2 region of the cardiac myosin rod as well as a mechanistic role of autoantibody in the disease pathogenesis. Li *et al.* (2006) recently built a model of cardiac myosin-induced myocarditis in Lewis rat, and showed that the myosin antibodies can not only react with the surface of cardiomyocytes, but also cross-react with the β-adrenergic receptor and subsequently induce cell signaling and cyclic AMP-dependent protein kinase (PKA) reaction which may finally lead to apoptosis in the myocardium. In addition, a further proof of mimicry is shown in Western blots that anti-cardiac myosin McAbs can react with the 67-kDa β-adrenergic receptor and the 200-kDa human cardiac myosin molecule and may contribute to the mechanism of antibodies against the β-adrenergic receptors during the course of the disease. And we are able to extrapolate our findings from the Lewis rat to human myocarditis and determine if antibodies against cardiac myosin and the β-adrenergic receptor were present in human disease and also could function as signaling antibodies. In support of this hypothesis, patients with myocarditis and dilated cardiomyopathy have elevated antibodies against cardiac myosin and immunosuppressive or immunoabsorption therapy can improve heart function in myocarditis patients (Cooper and Shabetai, 1995; Burgstaler *et al.*, 2007; Cooper *et al.*, 2007). This implies that the hypothesis above may bring pharmacotherapy of monoclonal antibody application in myocarditis to a new horizon. Although we have not identified the regions of cross reactivity between the β-adrenergic receptors and human cardiac myosin, studies are in progress with a human McAbs which recognizes both antigens and will be used to identify common structural regions of the molecules.

In addition, researches have indicated that calcium overload may lead to myocardial injury and damage. The reason for myocardial calcium overload in the present case is unknown. Possible pathogenetic mechanisms include an increase of endogenous catecholamines (Ferrans *et al.*, 1969; Fleckenstein *et al.*, 1971) in the final stage of myocarditis combined with the contribution from subsequent physiological or pathologic effects due to the autoimmune reaction with the β-adrenergic receptor recognized by the antibodies. NT4 peptides of myosin and streptococcal M protein are highly homologous in a structure with immunological properties. NT4 peptide of streptococcal M protein can induce myocardium inflammation, and is the major histocompatibility complex (MHC) type II restricted and $CD4^+$ T cell-dependent (Cunningham, 2004).

4.3 Monoclonal antibody therapy against apoptosis

Apoptosis is a programmed cell death under the control of genes, resulting in cell shrinkage and nuclear degradation. Apoptosis is involved in the pathogenesis of myocarditis and dilated cardiomyopathy.

Currently it has been shown that MYC, BCL-2,

p53, and FAS genes play an important role in the regulation of apoptosis. In myocarditis, apoptosis not only exits in morphology, but also proves that BCL-2 expression exists in the acute phase and BAX has high expression during the late stage.

Nussinovitch and Shoenfeld (2010) described a novel system to study fibrosis in myocarditis and the origins of the fibrosis in a mouse model, and found that fibrosis could be blocked by anti-transforming growth factor β (TGF-β) treatment. Mice expressing enhanced green fluorescent protein (EGFP) were used as donors of prominin-1^+ cells which may directly lead to fibrosis during the development of the chronic disease state in myocarditis and cardiomyopathy. Prominin-1^+ cells are precursors of fibroblasts in the bone marrow and once injected into hearts were shown to develop into fibroblasts and produce collagen in the presence of TGF-β (Cunningham, 2009). To prevent fibrotic changes in the heart, anti-TGF-β most likely affected the prominin-1^+ cells and prevented them from being transformed into fibroblasts by TGF-β and therefore producing fibrosis in the heart. Control of the fibrosis could be a turning point in preventing loss of function and end stage heart disease. These studies are directly applicable to human disease. TGF-β is a potential turning point that therapy may prevent the chronic and destructive progression to the irreversible end stage dilated cardiomyopathy with lowered ejection fraction and loss of function in the heart.

The c-Fos, a nuclear proto-oncogene, discovered as the cellular homologue of three distinct tumor viruses originated from mice and chicken (Im and Cp, 1988), has been particularly used in the description of the growth factor response pathway and molecular mechanism of various important cellular processes. These studies have provided important information on gene regulation in response to growth factors, and the function and interaction of transcription factors.

Indeed, c-Fos may have a protective function, including DNA repair, against harmful consequences of agents (Inada *et al.*, 1998). However, overexpression of c-Fos may lead to some diseases, such as cardiac ischemia-reperfusion, myocardial stunning, and heart failure (Corbucci, 2000; Itoh *et al.*, 2000; Patel *et al.*, 2000; Nelson *et al.*, 2002; Le *et al.*, 2005; Turatti *et al.*, 2005; Wang *et al.*, 2005; Sakai *et al.*, 2007; Aikawa *et al.*, 2008). As an example, the expression of Fos protein presents an apparent increase in rat models of myocardial stunning. Therefore, Fos may play a role in myocardial stunning for the reason that it has a close relationship to injury repair of the molecule (Aikawa *et al.*, 2008). In conclusion, the expression of Fos is mainly associated with cellular damage and subsequent death attributed to hypoxic-ischemic injury. In addition, studies on mechanism of the apoptosis induced by c-Fos suggested that c-Fos may play partially as a potent inducer of apoptosis in pro-B cells. The supposal that c-Fos may lead to apoptosis is further supported by findings that the induction of c-Fos expression is an inchoate event in many mammalian apoptosis cases (Colotta *et al.*, 1992; Wu *et al.*, 1993). Moreover, reduction of c-Fos activity by antisense oligonucleotides can prevent growth factor-deprived lymphoid cells from undergoing apoptosis. Since TNF-α and other cytokines rise apparently in VMC (Glück *et al.*, 2001; Calabrese *et al.*, 2004; Reifenberg *et al.*, 2007), isoproterenol, TNF-α and other cytokines induce expression of c-Fos oncogene (Haliday *et al.*, 1991; Emch *et al.*, 2001; Ono *et al.*, 2004; Takeshita *et al.*, 2005), Zhang *et al.* (2010) have made observations of abnormal expression of c-Fos in VMC, and in the cardiomyocyte of VMC mice they found an increase of the expression of c-Fos, and that c-Fos is able to produce AP-1 with products of c-jun gene. In addition, they have tried to use c-Fos McAb as an experimental treatment method to treat the VMC mice, and ultimately found a significant decrease of myocardial necrosis and cell infiltration. Fuse *et al.* (2000) observed, in patients with myocarditis, that the increasing of sFas and sFasL positively correlated with severity of myocarditis. Early detection of sFas and sFasL levels in patients with myocarditis can also be used to judge the prognosis of myocarditis. They also used anti-FasL McAb to treat acute myocarditis induced by CVB3, which not only reduced myocardial necrosis obviously, but also reduced IL-2, inducible nitric oxide synthase (iNOS) and CVB3 gene expressions in myocardial tissue. Thus the blocking of the Fas/FasL pathway prevents myocardial cell damage and improves the myocarditis patient prognosis and presents a feasible method of immunotherapy.

5 Conclusions

Development of monoclonal antibody technology has opened a new train of thoughts for clinical treatment of diseases. Monoclonal antibodies with high purity and strong specificity can accurately act on targets and have achieved definite progress in the treatment of VMC in rat models, and may supply an effective therapeutic approach to relieve the severity of myocarditis. However, several issues remain open. Since there was no complete reduction of the VMC symptoms following single autoantibody, the following factors may be considered as potential causes. Firstly, as the neutralization of the cytokine antibody biological activity was not quantified, the dosage of given McAb might not completely antagonize circulating autoantigen in vivo. Secondly, the biological activity, affinity, and/or potency of antibody in vivo are uncertain. Furthermore, other subsequent physiological or pathologic factors seem to compensate for the temporary deficiency of single autoantigen and provide an efficient effector cell recruitment. Loss of response to single autoantigen inhibition may also result from the induction of other pathways and thereby replace the initial contribution. So the technology on how to make a higher homologous and weak immunogenic humanized or human source antibody and the treatment mechanism of monoclonal antibodies are worthy of further studies. As the next step of our research we will work on to promote the clinical application of monoclonal antibody therapy to benefit patients with myocarditis and DCM.

Compliance with ethics guidelines

Li-na HAN, Shuang HE, Yu-tang WANG, Li-ming YANG, Si-yu LIU, and Ting ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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