Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular and Cellular Cardiology 38 (2005) 593–605
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Successful treatment of experimental autoimmune myocarditis by adenovirus-mediated gene transfer of antisense CIITA

Gang Cai, Jun Zhang, Li Liu, Qian Shen *

Department of Laboratory Diagnosis, Changhai Hospital, Second Military Medical University, 174 Changhai Avenue, Shanghai 200433, China

Received 13 October 2004; received in revised form 30 December 2004; accepted 21 January 2005

Available online 19 March 2005

Abstract

Experimental autoimmune myocarditis (EAM) has been used as a model for human myocarditis in relation to the autoimmune mechanism and proved as a T cell-mediated autoimmune disease. Interaction of T cell receptors (TCR) with its ligand peptide-MHC complex on APCs is critical for antigen-specific T cell activation under physiological and pathological conditions. CIITA is a transcriptional coactivator that functions as a key regulatory factor for MHC-II expression. To achieve effective inhibition of interaction of TCR and peptide-MHC-II complex, adenovirus vectors containing antisense CIITA were constructed and their effects in preventing EAM were examined. Ad-CIITA was injected intravenously into mice on days 0–2 or 14–16 after myosin immunization to study the preventive effects on EAM in the T cell activation phase or inflammatory phase. Disease severity was determined by the microscopic grade of heart check, concentration of plasma cTnI, and cellular and humoral immune responses on day 21. Results show that onset of EAM after Ad-CIITA treatment on days 0–2 was almost completely inhibited and antigen-specific lymphocyte proliferation was significantly reduced in adenovirus treatment group, which demonstrate that this adenovirus vector inhibit auto-responsive T cells activation and proliferation. Moreover, compared with EAM mice, even administered on days 14–16, the Ad-CIITA treated mice achieved significant reduction in disease severity. It indicates the therapeutic potential of blocking T cells activation by gene-transfer in myocarditis.

Keywords: CIITA; MHC-II molecule; Experimental autoimmune myocarditis; Adenovirus vector; Gene therapy; Antisense nucleic acid; T cell-mediated autoimmune disease; Immune response; Gene-transfer

1. Introduction

Myocarditis is a major cause of sudden death in people younger than 40 years of age [1] and often follows infection by coxsakievirus B3 [2]. The pathogenesis of myocarditis is not fully understood but there is substantial evidence suggesting the autoimmune responses to heart antigens, particularly cardiac myosin, after viral infection may contribute to the disease process [3].

An experimental model of autoimmune myocarditis (EAM) has been produced by immunization with cardiac myosin in susceptible rodent animals, such as Balb/c mouse. It is characterized by severe myocardial lesion and multinucleated giant cells infiltration. It has been reported that the pathogenesis of the tissue damage in human giant cell myocarditis (GCM) and viral myocarditis resembles that in EAM [4]. This model of myocarditis has also been shown to develop into postmyocarditic dilated cardiomyopathy (DCM) in the chronic phase, the histopathologic findings of which closely resemble those forms of human idiopathic DCM [5]. Therefore, we constructed an EAM derived from Balb/c mice to understand the mechanisms of myocardial injury and to develop an effective therapeutic strategy for myocarditis and DCM.

EAM is a T cell-mediated autoimmune disease because it can be transferred into naïve animals by injection of cultured spleen T cells from previously immunized syngeneic ones [6]. T cells require two distinct signals for their full activation [7]. The first signal is provided by the engagement of T-cell receptor (TCR) with major histocompatibility complex (MHC) plus peptides on antigen-presenting cells (APCs), and the second costimulatory signal is provided by engagement of one or more T-cell surface receptors with their ligands

* Corresponding author.
E-mail address: caigangsmmu@hotmail.com (Q. Shen).

0022-2828/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2005.01.009

on APCs [8]. MHC-IImolecules (MHC-II) plays a critical role in the induction of immune responses by presenting fragment of autoantigenic peptides to $CD4^+$ T lymphocytes, then resulting in activation of $CD8^+$ T lymphocytes and B lymphocytes. Moreover, under the status of autoimmune diseases, expression of MHC-II increases abnormally [9]. So, MHC-II was associated with autoimmune diseases. There are codominance and multiple allele for MHC-II which lead to their complicated polymorphism, so it is difficult to repress every MHC-II expression directly. MHC class II transactivator (CIITA) is the major rate-limiting factor for both constitutive and inducible MHC-II expression, and with rare exceptions, expression pattern of CIITA dictates most qualitative and quantitative aspects of MHC-II gene expression [10]. The cell type specificity, induction, and level of MHC-II expression are in the majority of situations determined by the expression of CIITA [11]. The gene encoding CIITA (*MHC2TA*) is controlled by three distinct and independent promoters referred to as p^-, pβ, and pχ. These promoters are spread out over a large (>12 kb) regulatory region and exhibit different cell type specificities [12]. pβ is used mainly in B cells [12]. IFN-γ-induced CIITA expression is mediated by pχ [13]. It previously showed that expression of the *MHC2TA* gene in dendritic cells (DCs) was primarily controlled by p^-, and to a lesser degree by pβ [12].

The goals of the present study are, therefore, to attenuate EAM by inhibiting the activation of auto-responsive T cells through down-regulating the expression of CIITA resulting in decreasing of expression of MHC-II. To achieve effective inhibition of CIITA expression, we constructed recombinant adenovirus Ad-CIITA containing the antisense *MHC2TA* gene fragment. We examined the effects of the adenovirally delivered antisense mRNA on the prevention of EAM shortly after the immunization (in the T-cell activation phase) and the initial inflammatory phase.

In this study, we demonstrated that: (1) the expression of MHC-IIwas enhanced in the heart of the EAM mice compared with normal mice; (2) both Ad-CIITA treatment on days 0–2 and delayed treatment on days 14–16 strikingly inhibited the onset and the ongoing EAM; (3) inhibition of expression of MHC-II by Ad-CIITA could inhibit antigen-induced spleen cell proliferation in vitro in dose-dependent manner. This study indicates that preventing the activation of T cells through down-regulating the expression of CIITA resulting in decreasing of the expression of MHC-II by Ad-CIITA has therapeutic potential for human myocarditis.

2. Materials and methods

2.1. Animals

Male Balb/c mice, 4–6 weeks of age at initiation of the experiments, were purchased from Shanghai experimental animal center (Shanghai, China) and feed in our animal facilities. During the period of experiment, all the investigation is conducted in conformity with principles about care and use animals.

2.2. Preparation of cardiac myosin

Cardiac myosin was prepared from the ventricular muscle of the porcine heart according to the methods of Murakami et al. [14], and used as an antigen.

2.3. Induction of autoimmune myocarditis

Male Balb/c mice were immunized subcutaneously twice with 200 μg of cardiac myosin in an equal volume of complete Freund's adjuvant (CFA, Sigma Chemical Co., St. Louis, MO) on days 0 and 7. The normal control mice were immunized with CFA and phosphate-buffered saline (PBS).

2.4. Experimental design

In preventive experiment, 5.0×10^8 plaque forming unit (PFU)/day × 3 days of Ad-CIITA or Ad-GFP serving as a control was injected intravenously (i.v.) on days 0–2 after immunization to study the preventive effects on EAM in the T-cell activation phase. On day 21 after the first immunization, these animals were sacrificed, blood samples were collected from caudal vein, and the hearts were removed, and divided for histology, biochemical examinations and recombinant adenovirus copies assay.

In therapeutic experiment, 5.0×10^8 plaque forming unit (PFU)/day × 3 days of Ad-GFP as a control, or Ad-CIITA was injected i.v. on days 14–16 after immunization to study the preventive effects on EAM in the inflammatory phase. On day 21 after the first immunization, these animals were killed, and investigation was taken as described above.

2.5. Virus preparation

The E1/E3-deleted recombinant adenoviruses Ad-CIITA were constructed with the AdEasy system (Stratgene, La Jalla, CA). Briefly, total RNA obtained from IFN-γ-stimulated mouse coelio-macrophage cells was reverse transcribed using Moloney murine leukemia virus polymerase (TaKaRa, DaLian, SD). Next, the 430bp fragment of CIITA cDNA (Gi: 6318321) was amplified by PCR using CIITA specific oligonucleotide primers (5′-CGACCATAGTCTGTGTGCCA-3′ and 5′-GCTTCCTGT GCTTTGAGTCC-3′) and subcloned into the clone vector pMD18-T (TaKaRa, DaLian, SD). A *Kpn*I/*Hin*dIII fragment was treated with restriction enzyme and cloned in reverse orientation into the same endonuclease site of pAdTrackCMV yielding the pAdTrackCMV-as-CIITA plasmids. These plasmids were cotransfected together with pAdEasy-1 into electrocompetent BJ5183 bacterial cells for homologous recombination. For generation of viral particles, the resulting plasmids were transfected into 293 cells using the Lipofectamine2000 reagent (Gibco BRL, Rockville, MD). The resulting adenoviral vectors, denoted Ad-