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Recombinant idiotypic TCRβ chain immunization in mice generates antigen specific T cell response

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Abstract

Vaccination remains the most cost-effective means of preventing infectious diseases. Success of vaccination depends on generation of effective memory response. Understanding the mechanism of generation and maintenance of immunological memory would help in the design of rational vaccines. T lymphocytes play a central role in the generation of protective immune response against many microbial infections. A hypothesis known as relay hypothesis was earlier proposed, which explains the maintenance of immunological memory through interaction of idiotypic and anti-idiotypic lymphocytes. In the present study, we have shown that immunization with a model antigen, chicken ovalbumin specific T cell receptor beta chain (idiotypic TCR) generates TCR specific antibody and anti-idiotypic T cell responses as well as ovalbumin specific T cell response. We further show that boosting of ovalbumin primed mice with ovalbumin specific idiotypic TCRβ DNA or TCRβ protein gives memory response for ovalbumin. This study provides experimental evidence for perpetuation of immunological memory through idiotypic network interactions.

Keywords: Anti-idiotypic T cells; DNA vaccine; T cell receptor; T cell memory; Relay hypothesis

1. Introduction

Immunological memory is the hallmark of acquired immune response. It results from the clonal expansion and differentiation of antigen specific lymphocytes. Memory lymphocytes confer immediate protection in peripheral tissues and mount recall responses to antigens in secondary lymphoid organs. Vaccines for many pathogens, including AIDS, malaria and tuberculosis are either ineffective or un-available. Many diseases require a strong component of cellular immune response for their control. It has been demonstrated that plasmid DNA vaccines can induce both humoral and cellular immune responses in a variety of murine and primate disease models (Ulmer et al., 1993; Gurunathan et al., 2000). DNA vaccine provides several potential advantages over other currently used vaccines: (a) It mimics the effects of live attenuated vaccines in its ability to induce major histocompatibility complex I restricted $CD8^+$ T cell response, which may be advantageous compared to conventional protein based vaccines, while mitigating some of the safety concerns associated with live vaccines. (b) It can be manufactured in relatively cost-effective manner and stored with relative ease for long periods of time, eliminating the need for a "cold chain".

DNA vaccine has been examined for control of viral (Kucerova, 1998), bacterial (Ha et al., 2005), parasitic (Ivory and Chadee, 2004), autoimmune (Matsumoto, 2000; Miyakoshi et al., 2003) and neoplastic diseases (Mizutani et al., 2004). It has been demonstrated that immunization with single chain TCR fusion protein or TCRβ chain alone induces anti-clonotypic immunity and protection against T cell lymphoma (Thirdborough et al., 2002; Zhang et al., 2004). This was proposed to be due to the generation of anti-idiotypic T cell response against T cell lymphoma. Anti-idiotypic T cell immune response has been generated after immunization with irradiated idiotypic T cells or peptides derived from idiotypic determinants of TCR, which confer protection against self-reactive or autoimmune T cells (Cohen et al., 2004; Zang et al., 2003). Generation of T cells response against idiotypic and anti-idiotypic antibody have been proposed to help in the maintenance of immunological memory (Nayak et al., 2001, 2005).

The structural diversity in TCR α and β chains is generated by somatic recombination of V(D)J genes segments and

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provides unique idiotypic determinants expressed clonotypically by T cells (Sebzda et al., 1999). These idiotypic determinants can serve as targets for active anti-idiotypic T cell immune response. As proposed earlier, this anti-idiotypic T cell can carry the peptidomimic and perpetuate memory response in absence of antigen (Nayak et al., 2001, 2005).

To test whether immunization with anti-idiotypic TCR can give antigen specific T cell memory response, we have cloned ovalbumin specific TCRβ chain gene (Fujio et al., 2000) into prokaryotic and eukaryotic expression vectors. The recombinant TCRβ protein was expressed in bacterial system and purified. Upon TCRβ DNA immunization, anti-idiotypic T cell response was generated in mice. We show that boosting with TCRβ DNA or TCRβ protein induces memory response against ovalbumin. This is the first demonstration of anti-idiotypic T cell inducing antigen specific T cell memory response.

2. Materials and methods

2.1. Plasmids

cDNA of TCRβ chain, derived from $CD4^+$ T cells of DO-11.10 transgenic mice was cloned in the retrovirus pMX vector (Fujio et al., 2000; Kitamura et al., 2003). This ovalbumin specific TCRβ chain containing pMX plasmid vector (pMX-DOTBE) was a generous gift from Dr. T. Kitamura, University of Tokyo, Japan. pRSET-B and pCDNA-3.1 (*myc-his*) vectors were obtained from Invitrogen (USA). All the restriction enzymes and ligase were purchased from the MBI Fermentas (Germany). Ligation and restriction digestion reactions were performed according to manufacturer's instruction.

2.2. Animals and cell lines

Balb/c mice were bred and maintained in the Central Animal Facility, Indian Institute of Science, Bangalore, India. Six- to eight-week-old male mice were used in all the experiments. All the experiments were performed according to institutional ethical committee guidelines. P815 ($H2^d$ haplotype), a mastocytoma cell line was maintained in RPMI-1640 medium supplemented with 10% FBS (Life Technology, USA).

2.3. Construction of recombinant TCRβ chain plasmids

pMX-DOTBE plasmid was digested with *Eco*RI enzyme. TCRβ DNA fragment (976 bp) was released and gel eluted. Subsequently, it was cloned in pRSET B vector at *Eco*RI site. Ligation was confirmed by release of 976 bp insert after digestion with *Eco*RI enzyme. The orientation of the TCRβ was confirmed by digestion with *Hin*dIII. This recombinant TCRβ containing plasmid is named as pR-DOTBE.

The *Eco*RI fragment of TCRβ DNA was cloned into pCDNA3.1 (*myc-his*) plasmid at *Eco*RI site. The orientation of the insert DNA was confirmed by digestion with *Hin*dIII. This recombinant plasmid is named as pC-DOTBE. The P815 cells were transfected with pC-DOTBE plasmid. The expression of TCRβ chain in P815 cells was confirmed by Western blot using anti-histidine antibody and shows expression of TCRβ protein in pC-DOTBE plasmid transfected P815 cells but not in vector transfected cells. This confirms the expression of TCRβ protein in eukaryotic cells from pC-DOTBE plasmid. *E. coli* DH5α strain was transformed and endotoxin free plasmid was purified in bulk using Qiagen Plasmid Maxi kit (Qiagen, Germany) and used for immunization of animals.

2.4. Expression and purification of TCRβ protein in E. coli

E. coli BL-21 (DE3) strain was transformed with pR-DOTBE plasmid. Single transformed colony was inoculated into 5 ml LB medium (HiMedia, India) containing 100 μg/ml ampicillin and incubated overnight at 37 °C in an orbital shaker. LB medium (500 ml) containing 100 μg/ml ampicillin was inoculated with 5 ml of overnight culture and grown to an $OD_{600\,nm}$ of 0.5 in a orbital shaker incubator at 200 rpm at 37 °C. TCRβ expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.2 mM at 37 °C for 5 h. Cell extracts were made and the TCR protein was purified from the inclusion body according to the procedure of Razeghifard (2004).

2.5. Ovalbumin immunization

Six- to eight-week-old Balb/c mice (three mice in each set of experiments) were immunized with ovalbumin (50 μg/mouse) in Freund's complete adjuvant, subcutaneously. Mice were boosted with ovalbumin (50 μg/mouse) in Freund's incomplete adjuvant, subcutaneously.

2.6. DNA immunization

Six- to eight-week-old Balb/c male mice were immunized with pC-DOTBE plasmid (100 μg/mouse) in 50 μl of PBS by intra-muscular injection into bilateral musculus quadriceps femoris of mice. After 10 days, mice were boosted with pC-DOTBE plasmid or purified recombinant TCRβ protein (50 μg/mouse) in Freund's incomplete adjuvant. After 1 week of booster, splenocytes and sera from these mice were collected. Humoral responses were monitored by ELISA, and cellular response by in vitro re-stimulation with bacterially expressed purified TCRβ protein.

2.7. Indirect ELISA

Each well of 96 well ELISA plates (BD Falcon, USA) were coated overnight with bacterially expressed purified TCRβ protein (300 ng/well) in PBS at 4 °C. Plates were washed three times with PBST (PBS + 0.1% Tween 20). Wells were blocked with 3% bovine gelatin for 1 h at 37 °C. Plates were washed three times and incubated with the immunized mouse serum at different dilutions for 1 h at 37 °C. Plates were washed three times and incubated with donkey anti-mouse IgG-POD antibody (Jackson ImmunoResearch Laboratories, USA) for 1 h. Plates were washed five times with PBST and color was developed using