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Immunogenicity of Leishmania Donavani Centrin-3 Vaccines

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Abstract Leishmaniasis is a parasitic protozoal disease affecting humans and animals with phlebotomine sand flies as intermediate vectors. There is no effective vaccine in use against this parasite and production relies on finding potent immunogenic antigens with long lasting memory response. As part of searching for new Leishmania antigens of a potential vaccine application, the immunogenicity of L. donovani centrin-3 (Ldcen-3) was investigated in a Balb/c model. Ldcen-3 is a calcium binding protein that has been shown to be involved in duplication and segregation of the centrosome in higher and lower eukaryotes. The Ldcen-3 gene was cloned in various vectors and coated on gold particles for gene gun immunisation. Significant protection was induced by immunisation with 1μg DNA of pcDNA3.1-Ldcen-3 or pCRT7/CT-TOPO-Ldcen-3 constructs. Protection against challenge with live parasite was vector dependent where better protection was induced by pCRT7/CT-TOPO-Ldcen-3. Splenocytes from Balb/c mice immunised with pcDNA3.1-Ldcen-3 or pCRT7/CT-TOPO-Ldcen-3 had a potent CTL response against DC targets loaded with SLA or tumour cells transfected with Ldcen-3 plasmid construct.

Keywords: *immunogenicity, leishmania, centrin, vaccine*

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1. Introduction

Immunisation with plasmid DNA encoding Leishmania antigens represents a promising approach to vaccination against Leishmaniasis as it induces both humoral and cell mediated immune responses and results in long lasting immunity [1]. Therefore, DNA vaccination could potentially treat and prevent Leishmaniasis. Many vaccine strategies have been pursued, including the use of whole cell lysate, killed, virulent or irradiated parasite [2]. Leishmania DNA vaccines and purified or recombinant parasite antigens have also been tested. Most of the studied antigens have so far shown a limited degree of effectiveness as a potential vaccine in animal models but little or no protection in humans. New antigen discovery strategies are essential to identify new antigens with potential as a novel vaccine candidate.

The immunogenicity of centrin genes have not been previously studied and very little is known of their biology in Leishmania. A DNA-encoding N-terminal domain of the proteophosphoglycan (PPG) gene, which is a surface-bound protein in both promastigotes and amastigotes, was investigated as a vaccine in hamsters against challenge with L. donovani. A protection efficiency of about 80% was observed in vaccinated hamsters with more than 6 months survival after challenge. The efficacy was supported by a surge in inducible nitric oxide synthase, IFN-γ, TNF-α, and IL-12 mRNA levels along with down-regulation of TGF-β, IL-4, and IL-10. The level of Leishmania specific IgG2 was also increased which was indicative of an enhanced cellular immune response. It was concluded that the N-terminal domain of L. donovani PPG is a potential DNA vaccine against visceral Leishmaniasis [3].

Centrins are cytoskeletal calcium binding proteins that are localized in the microtubule organising centre (MTOC) of eukaryotic cells [4]. There are a number of eukaryotic centrin genes that have been identified, including four genes identified in mice, and three in humans [5,6]. The recently completed genome database for two trypanosomatids, i.e., Trypanosoma brucei, the causative agent of African sleeping sickness and Leishmania, have revealed five centrin genes in this group of organisms [7]. The functions of some of the centrins have been identified, for example, one group of centrins, which includes: C. reinhardtii centrin, Paramecium centrins 2 and 3, mouse centrins 1 and 2 and human centrins 1 and 2, are involved in centrosome and basal body segregation [8,9]. The other group containing Leishmaniacentrin-1, yeast centrin, mouse centrin-3 and human centrin-3, plays a role in centrosome and basal body duplication [10,11]. The N-terminal non-conserved domain of centrins, which is variable in length, is considered to be responsible for the functional diversity of centrins [6,12]. L. donovani centrin 3 (Ldcen-3)has a significantly smaller N-terminal region compared to centrins from other species [13].

The Ldcen3 gene is 100% homologous in L.donovani, L. major and L. mexicana. Therefore, the use of a DNA vaccine to stimulate an immune response against this protein could represent a novel approach to immunise humans against more than one species of the parasite. A DNA vaccine encoding Ldcen3 could offer protection against both visceral and cutaneous Leishmaniasis because of heterogeneity in DNA sequence to that of human centrin-3 Figure 1.

Knocking out the centrin-3 (Ldcen-3) gene reduced the growth rate of both promastigotes and amastigotes, and reduced survival in human macrophages in vitro [13,14]. Other studies showed that dominant negative expression of centrin proteins by parasites could result in reduced survival in macrophages in vitro or in reduced virulence in mice in vivo [15].

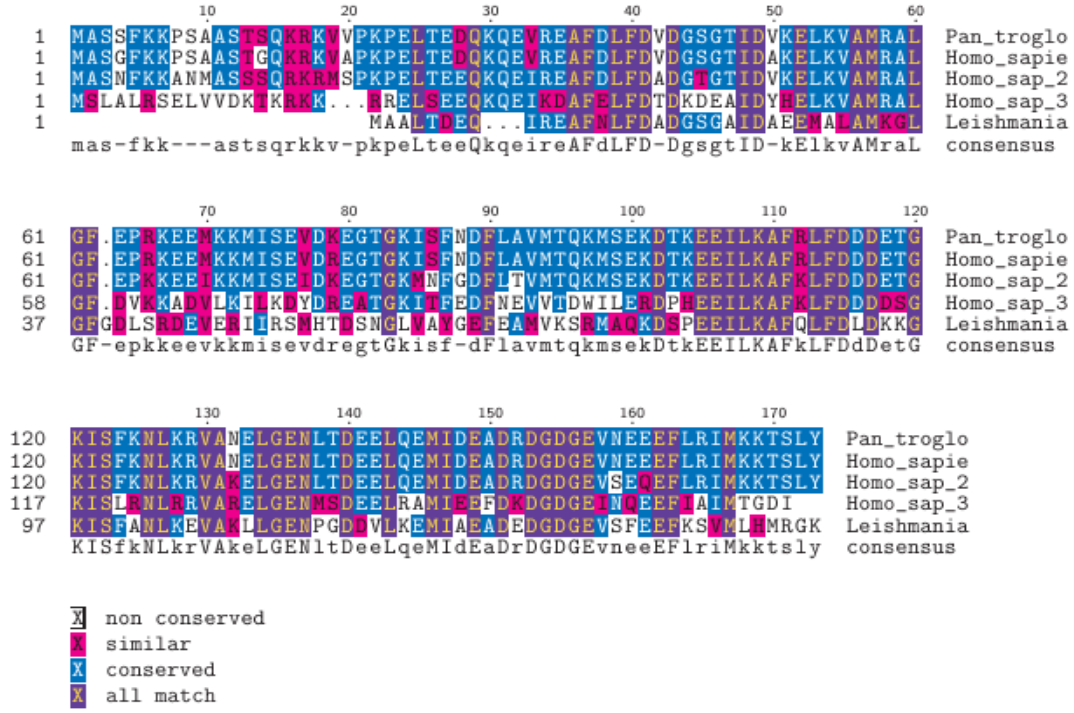

Figure 1. The amino acid sequence of Ldcen-3 compared with human centrins

2. Methods

In the present study, the immunogenicity of Ldcen-3 was investigated in a Balb/c L. mexicana model, using a gene gun to release a plasmid DNA construct coated on gold particles. The gene gun fires DNA coated gold particles at high velocity directly into epidermal cells, which consist of skin cells, Langerhans cells (LC) and dermal dendritic cells (DC). Inside the cell, plasmid is transported to the nucleus, the encoded gene is transcribed and the protein is subsequently produced, processed into peptides by host proteases and then presented in the context of MHC class I antigen which then stimulates CD8+ T cells [16,17]. DC directly transfected with DNA vaccine can prime CD8+ cells by presenting the DNA encoded antigen via MHC class I. Immature DC can endocytose soluble proteins and debris from apoptotic transfected cells and express the coded antigen through MHC class I or MHC class II following differentiation into mature DC. Thus, a DNA vaccine can be effective in the stimulation of both CD8+ T cells and CD4+ T cell populations. The ability of DCs to present extracellular antigens into MHC class I and MHC class II is known as cross priming. Accordingly, DCs play an important function in the induction of both humoral and cell mediated immunity following DNA vaccination.

3. Results

3.1. Confirming pCRT7/CT-TOPO as a Mammalian Vector

3.1.1. Sub Cloning of LacZ into pCRT7/CT-TOPO

To provide evidence that pCRT7/CT-TOPO-Ldcen-3 could transfect and express genes of interest in mammalian cells, pCRT7/CT-TOPO-Ldcen-3 was constructed incorporating the lacZgene as a marker and was used to transfect a mammalian cell line Figure 1. Briefly, the lacZ gene was cut from pcDNA3.1myc-His lacZ (-) vector from both sides using XbaIand Hind III restriction enzymes. The digested fraction was separated by gel electrophoresis (1.5 %). The pCRT7/CT-TOPO vector was also digested using the same restriction enzymes Figure 2-A, B, C&D. The Ldcen-3 was extracted from the gel and inserted in pcDNA 3.1(-) and then the lacZ gene and the empty pCRT7/CT-TOPO vectors were ligated using a DNA ligase enzyme. The pCRT7/CT-TOPO-lacZ vector was transfected in CT 26 cells to establish the expression ability in mammalian cells; a suitable CT 26 clone 25 (CT 26-lacZ) mouse tumour cell line stably transfected with lacZ gene, was used as a positive control.

3.1.2. Transfection of CT26 Cells with pCRT7/CT-TOPO-lacZ

β-galactosidase, an important reporter gene encoded by the lacZ gene, is commonly used for monitoring transfection efficiency in mammalian cells. The β-galactosidase staining kit was used to determine the expression of lacZ following transient or stable transfection of plasmids encoding lacZ. β-galactosidase catalyzes the hydrolysis of X-gal producing a blue colour. Transfected CT26 withpCRT7/CT-TOPO-lacZwas examined under a light microscope for the development of blue stain, which successfully produced a blue colour when compared to control cells CT26-lacZ indicating the ability of pCRT7/CT-TOPO to express lacZ in mammalian cells Figure 3.

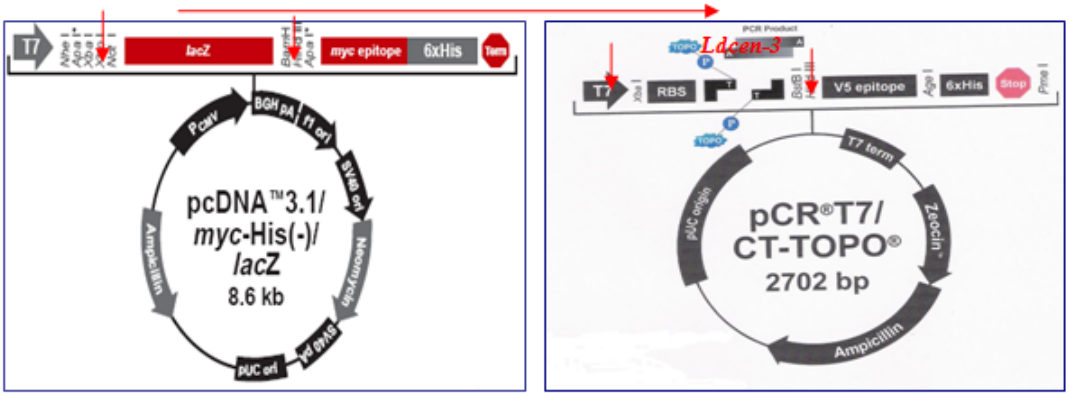

Figure 2A. Map representing pCRT7/CT-TOPO and pcDNA 3.1 myc-His/lacZ: pCRT7/CT-TOPO vector containing -Ldcen-3 gene and pcDNA 3.1 myc-His LacZ (-) to sub cloneLacZ in pCRT7/CT-TOPO

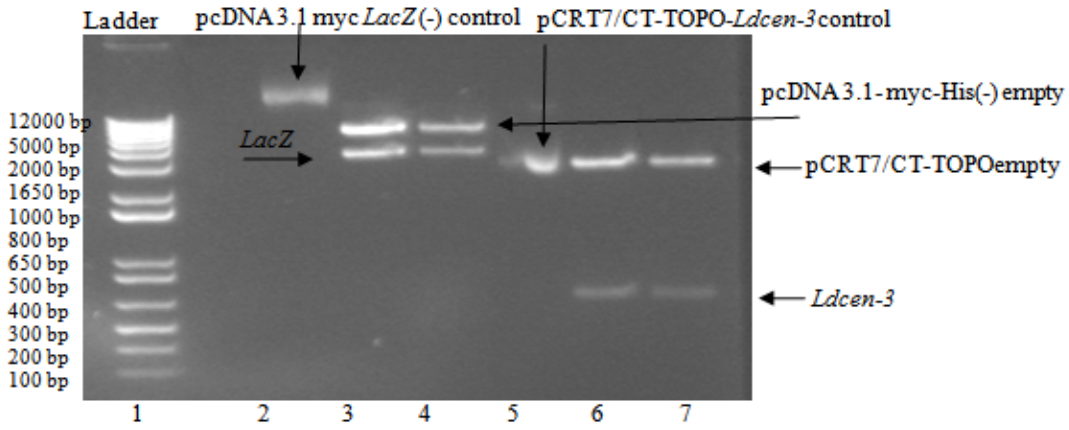

Figure 2B. Digestion of pcDNA 3.1 and pCRT7/CT-TOPO: Both plasmids were cut by XbaI and Hind III **restriction enzymes**: 1- ladder; 2- pcDNA 3.1 myclacZ (-); 3, 4-the above band is the linear – empty pcDNA 3.1 myc-His and lower band islacZ; 5, pCRT7/CT-TOPO-Ldcen-3 control; 6, 7, above band is the linear empty vector pCRT7/CT-TOPO and lower band is Ldcen-3

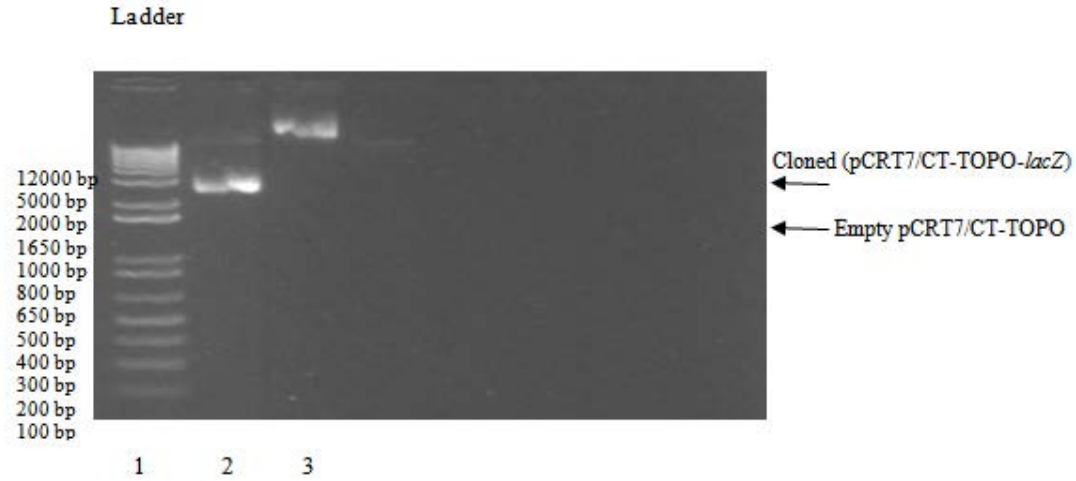

Figure 2C. Sub cloning of lacZ into the pCRT7/CT-TOPO vector: The lacZ gene and the digested pCRT7/CT-TOPO vector were collected, purified and ligated using a DNA ligase enzyme, 1- 1kd ladder; 2- empty pCRT7/CT-TOPOand 3- pCRT7/CT-TOPO-lacZ (maps and vectors were obtained from invitrogen)

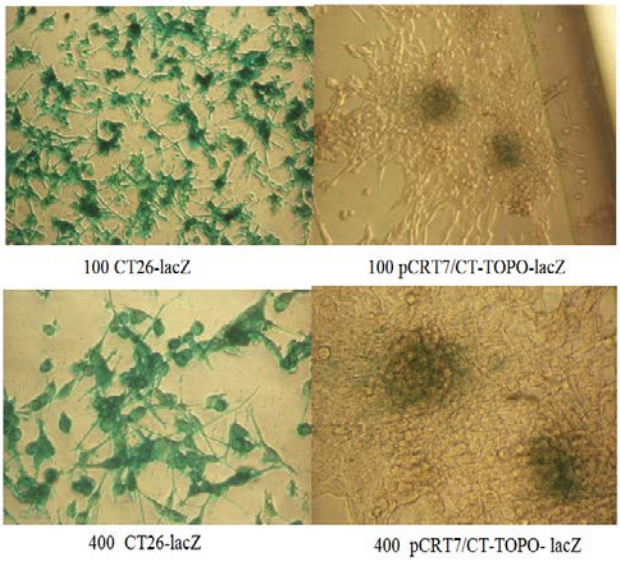

Figure 3. Expression of β-gal in CT26 transfected with pCRT7/CT-TOPO-LacZ CT26 cells transfected with a pCRT7/CT-TOPO-LacZ vector were washed twice with PBS and then fixed for 15 minutes with glutaraldehyde. Cells were then stained with X-gal for overnight to test the expression of pCRT7/CT-TOPO in mammalian cells. Blue colour staining indicates the expression of lacZ; suitable CT26-clone 25, (a stable transfectant with high expression of B-galactosidase) was used as a positive control

3.2. Subcloning of Leishmaniadonovani Centrin-3 (Ldcen-3) into pcDNA3.1

3.2.1. Confirmation of the Presence of Ldcen-3 by PCR

The pCRT7/CT-TOPO-Ldcen-3 vector was bulked up to obtain sufficient quantities of the plasmid and PCR was used to confirm Ldcen-3 presence using two primers designed for Ldcen-3 (541bp), Ldcen-3F, forward 5`AGA GGC ATT CGT GTT CG-3` and Ldcen-3R, reverse 5`AGG TTG ATC TCG CCA TCT TGA -3` Figure 4.

To determine the sequence of the Ldcen-3 gene, the DNA sample along with two primers that were used for the PCR amplification were sent to MWG-biotech.com for sequencing. This confirmed the presence of the pCRT7/CT-TOPO-Ldcen-3 gene insert.

3.3. Subcloning of Ldcen-3 into pcDNA3.1 (-)

In order to adopt a widely used mammalian vector for DNA immunisation and also to transfect CT26 tumour cells it was decided to sub-clone the Ldcen-3 gene into a pcDNA3.1 (-) vector Figure 5, which contained a mammalian selectable marker antibiotic gene. Ldcen-3 was cut from both sides by XbaI and Hind III restriction enzymes from the pCRT7/CT-TOPOvector. The digested fractions were separated by gel electrophoresis Figure 6A. The pcDNA3.1 (-) vector was also cut using the same restriction enzymes. The Ldcen-3 gene and the digested pcDNA 3.1 (-) vectors were then ligated using a DNA ligase enzyme. The presence of the Lcen-3 gene in the pcDNA3.1(-) vector was determined by restriction enzyme digestion Figure 6B and PCR amplification Figure 6C using forward and reverse primers Ldcen-3F 5`AGA GGC ATT CGT GTT CG-3`andLdcen-3R, reverse5`AGG TTG ATC TCG CCA TCT TGA -3`.

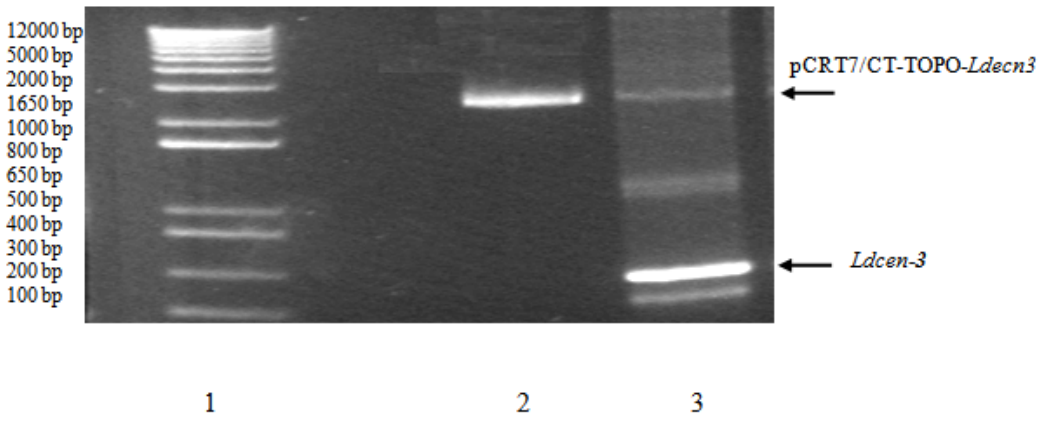

Figure 4. Confirmation of the presence of Ldcen-3 by PCR: The presence of Ldcen-3 was confirmed by PCR amplification using 5`AGA GGC ATT CGT GTT CG-3` forward and 5`AGG TTG ATC TCG CCA TCT TGA -3`reverse primers 1- ladder, 2- pCRT7/CT-TOPO-Ldecn3 as a control and not PCR result, 3- Ldcen-**3 (**PCR product)

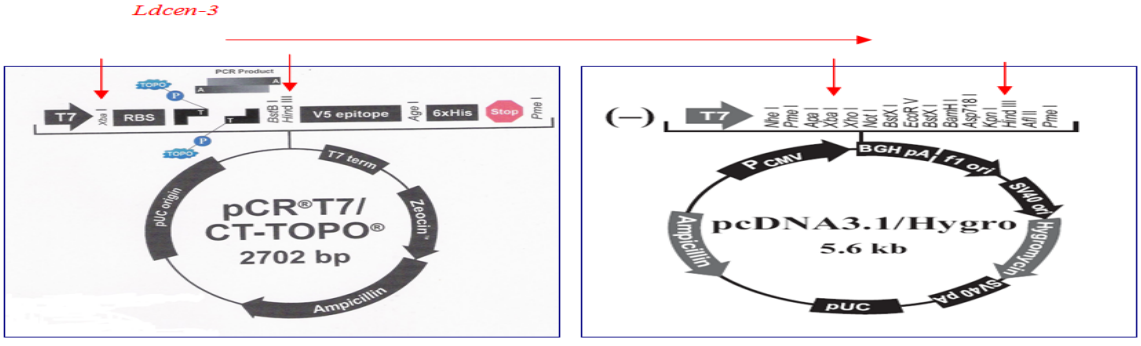

Figure 5. Map representing pCRT7/CT-TOPO-Ldcen-3 and pcDNA 3.1(-) vectors

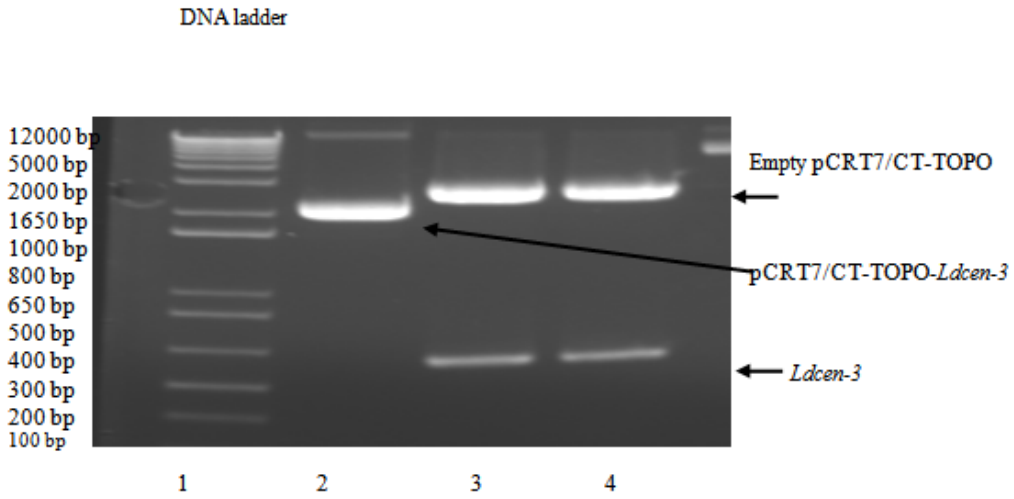

Figure 6A. subcloning of Ldcen-3 into pcDNA 3.1 (-) vectors: pCRT7/CT-TOPO-Ldcen-3 was cut by XbaI and Hind III restriction enzyme 1- ladder; 2- pCRT7/CT-TOPO–Ldcen-3, 3,4-the above band is a linear empty pCRT7/CT-TOPO and lower band is Ldcen-3

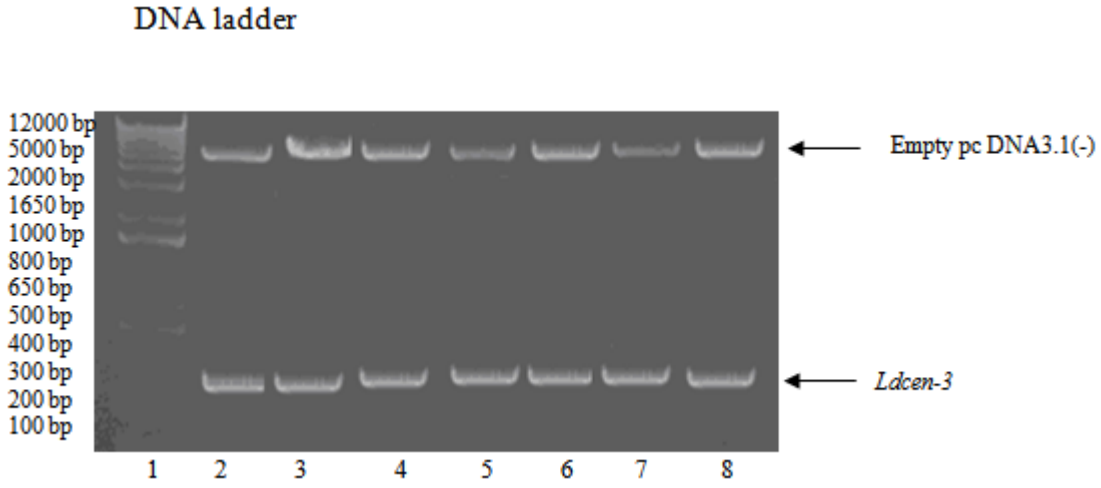

Figure 6B. Confirmation of the presence of Ldcen-3 in pcDNA3.1 (-): Restriction digestion with the same enzymes (Hind III and XbaI restriction enzymes), 1-ladder, 2-8 the upper bands are linear empty pcDNA3.1 (-) and the lower bands are Ldcen-3 after digestion of pcDNA3.1 (-)-Ldcen-3 with Hind III and XbaI

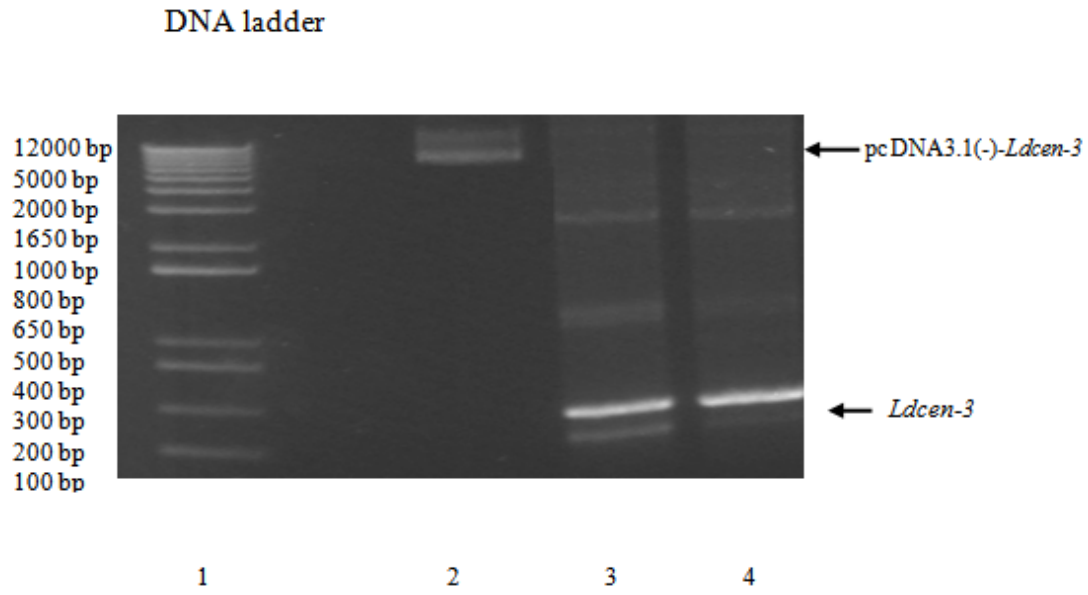

Figure 6C. Confirmation of the presence of Ldcen-3 in pcDNA3.1 (-) by PCR: The presence of Ldcen-3 gene was confirmed by PCR using Ldcen-3 forward primer F 5`AGA GGC ATT CGT GTT CG-3` and the Ldcen-3 reverse primer R, 5`AGG TTG ATC TCG CCA TCT TGA-3`, 1-DNA ladder, 2-empty pcDNA3.1 (-)-Ldcen-3 and 3, 4 Ldcen-3

3.3.1. Construction of pCRT7/CT-TOPO Empty Vector

In order to produce a pCRT7/CT-TOPO empty vector to be used as a negative control in DNA vaccination and protection studies, the Ldcen-3 gene was cut and removed from this vector. The Ldcen-3 gene was cut out from the vector by digestion with XbaI and Hind III restriction enzyme and the product was run into an agarose gel. The band related to the vector was extracted from the gel Figure 7. Both free ends of the vector that resulted from digestion were then ligated to each other by the ligase enzyme. The absence of the Ldcen-3 gene in the empty vector was confirmed by PCR using Ldcen-3 primers.

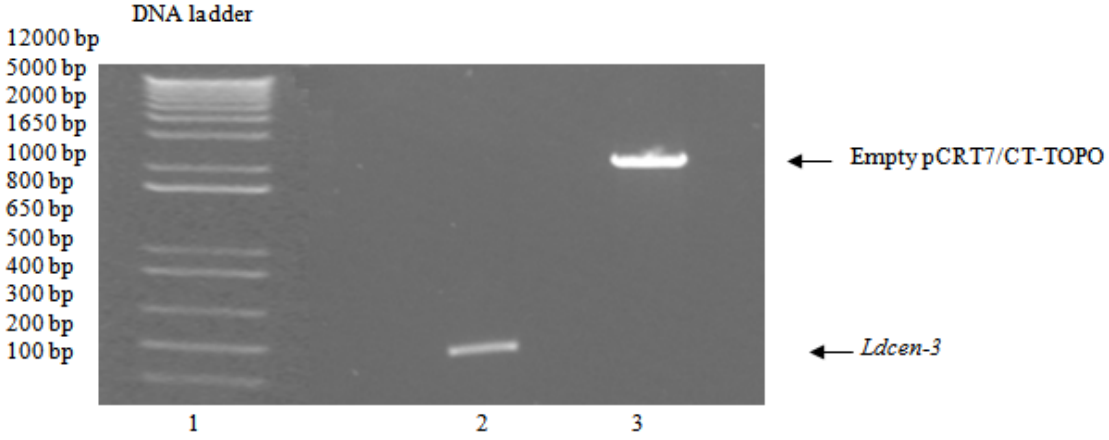

Figure 7. Production of empty pCRT7/CT-TOPO vector

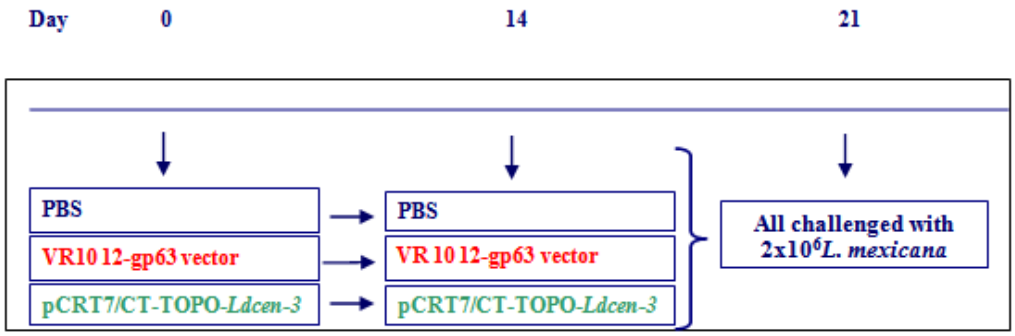

Figure 8A. Immunisation protocol

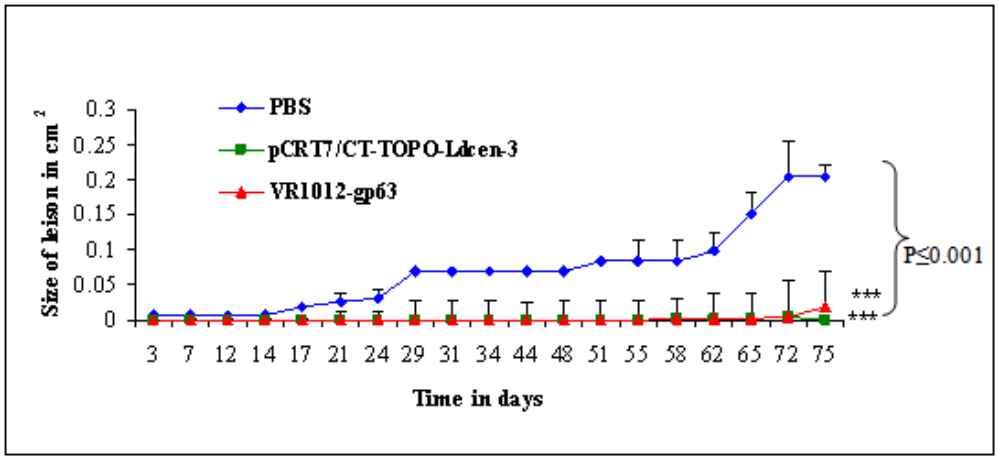

Figure 8B. Protection induced by immunisation with pCRT7/CT-TOPO- Ldcen-3 construct

3.4. Immunogenicity of Ldcen-3

3.4.1. Protection induced by Immunisation with pCRT7/CT-TOPO-Ldcen-3 Plasmid Construct

To determine the immunogenicity of Ldcen-3 (L. donovanicentrin-3), a pCRT7/CT-TOPO-Ldcen-3was used as a DNA vaccine in a Balb/c mouse model. L. mexicana gp63 construct (VR1012-gp63) was used as a positive control since this gene (L. mexicana gp63) was shown to induce strong immunity by DNA-gene gun immunisation [17]. The results Figure 8 clearly demonstrated that mice immunised with Ldcen-3 or gp63 were significantly protected against challenge with live parasites, 5 out of 6 mice were lesion free in Ldcen-3 or gp63 groups.

3.4.2. Protection Induced by Immunisation with pCRT7/CT-TOPO- Ldcen-3 and pcDNA3.1-Ldcen-3 Plasmid Construct

The immunogenicity of Ldcen-3 cloned in two different vectors was investigated to confirm the immunogenicity to Ldcen-3. Balb/c mice were immunised by gene gun with 1µg of pcDNA3.1-Ldcen-3. pCRT7/CT-TOPO-Ldcen-3, empty pcDNA3.1, and empty pCRT7/CT-TOPO, PBS was used as a control. The results show that a significant protection was induced by immunisation with 1µg Ldcen-3 constructs which was vector dependent since pCRT7/CT-TOPO-Ldcen-3 (4 out of 6 free of lesion) induced better protection than pcDNA3.1-Ldcen-3 (3 out of 6 free of lesion) Figure 9. The empty pCRT7/CT-TOPO (0/6) vectors did not protect mice from challenge. Although immunisation with empty pcDNA 3.1 vector slowed down lesion development in immunised mice.

3.4.3. CTL Activity in Balb/c Mice Immunised with pcDNA3.1 (-)-Ldcen-3 and pCRT7/CT-TOPO-Ldcen-3 by Gene Gun

To evaluate the role of cytotoxic T cells in immunity to Leishmania, a standard 4-hour ^{51}Cr-release cytotoxicity assay was used to assess the ability of L. mexicana Ldcen-3construct to generate specific cytotoxic T lymphocytes by immunisation. Balb/c mice were immunised with pcDNA3.1 (-)-Ldcen-3 and pCRT7/CT-TOPO-Ldcen-3. Splenocytes were harvested from immunised mice and cultured in vitro for 5 days together with blasts cells pulsed with LPS and SLA2 (see chapter 2 methods). On day 5, the splenocyte cells were used as effectors in standard 4-hour ^{51}Cr-release cytotoxicity assay against non-adherent DCs loaded with SLAs and DCs alone as target. Splenocytes from Balb/c mice immunised with pcDNA3.1 (-)-Ldcen-3 or pCRT7/CT-TOPO-Ldcen-3 induced significant CTL activity compared with empty vectors Figure 10.

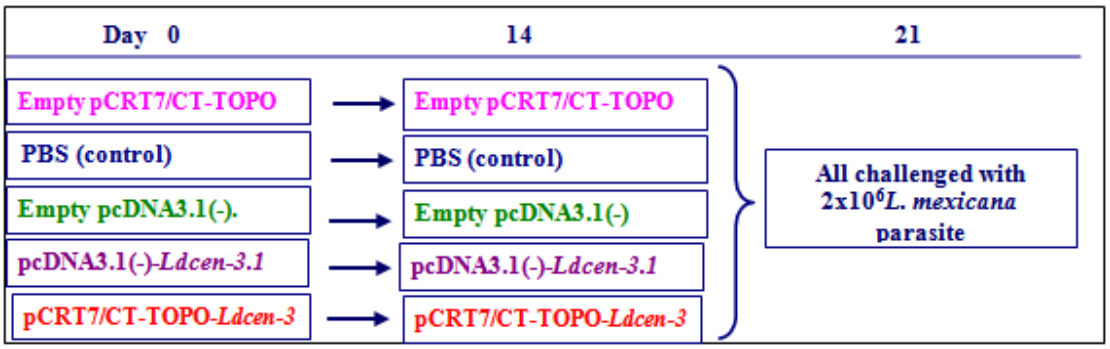

Figure 9A. Immunisation protocol

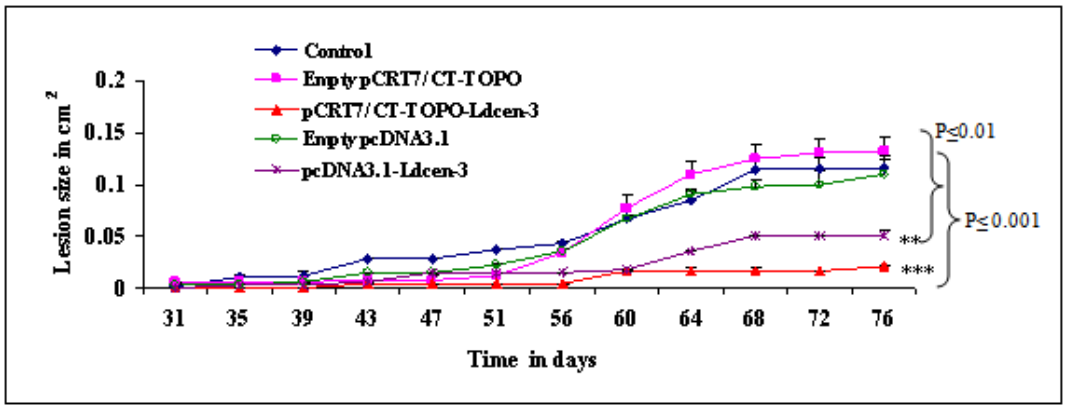

Figure 9B. Protection induced by immunisation with pCRT7/CT-TOPO- Ldcen-3 and pcDNA3.1-Ldcen-3

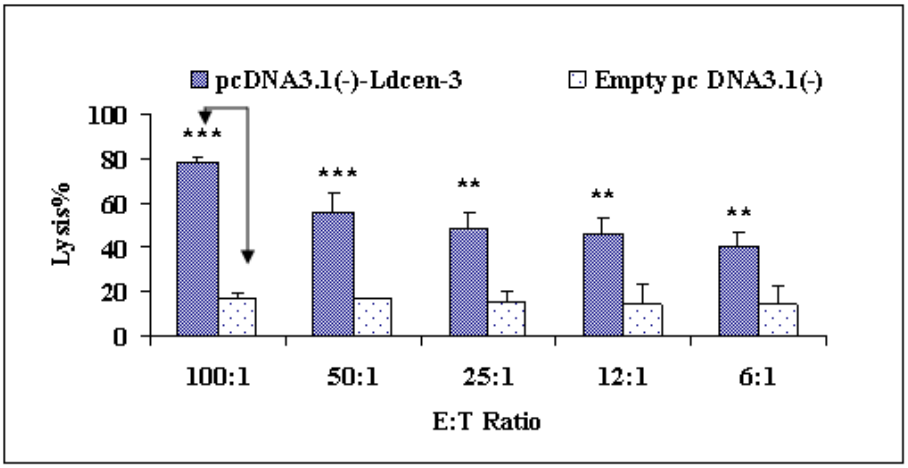

Figure 10A. CTL activity of Balb/c mice immunised with 1µg pcDNA3.1-Ldcen-3 or mice immunised with 1µg empty pcDNA 3.1(-) by gene gun

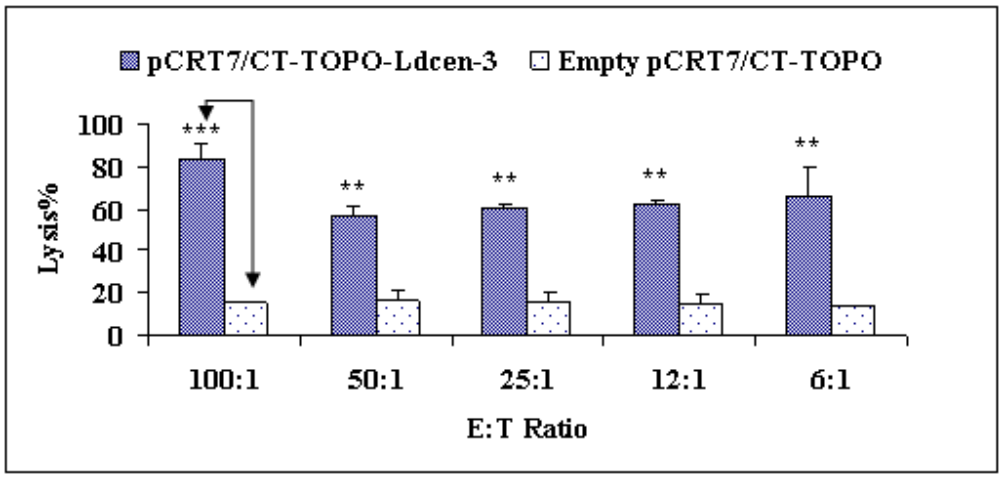

Figure 10B. CTL activity of Balb/c mice immunised with pCRT7/CT-TOPO-Ldcen-3 or immunised with empty pCRT7/CT-TOPO by gene gun. Splenocytes were stimulated with SLA for 5 days and used as effector cells in a standard 4-hour cytotoxicity assay against DCs pulsed with SLA.The graph represents 4 mice in 2 independent experiments P**≤0.01, P***≤0.001 by T test

3.4.4. Transfection of CT26 Cells with pcDNA3.1 (-)-Ldcen-3

In this study, CT26 tumour cells were transfected with pcDNA3.1 (-)-Ldcen-3 DNA using lipofectamine 2000, according to the manufacturer's instructions, to investigate CTL activity against targets expressing Ldcen-3 antigen. The presence of the Ldcen-3 gene was determined in the stable transfected cells by RT-PCR using forward and reverse primers: Ldcen-3F 5`AGA GGC ATT CGT GTT CG-3` andLdcen-3R, reverse5`AGG TTG ATC TCG CCA TCT TGA -3`. The transfected CT26-Ldcen-3 clearly shows a strong band for Ldcen-3. Also, for unknown reasons, non-transfected CT26 cells always showed a faint band when tested with the primers, which is not a specific band compared with transfected CT26 this was also observed when CT26 cells was transfected with L. mexicana gp63 plasmid construct [17] Figure 11.

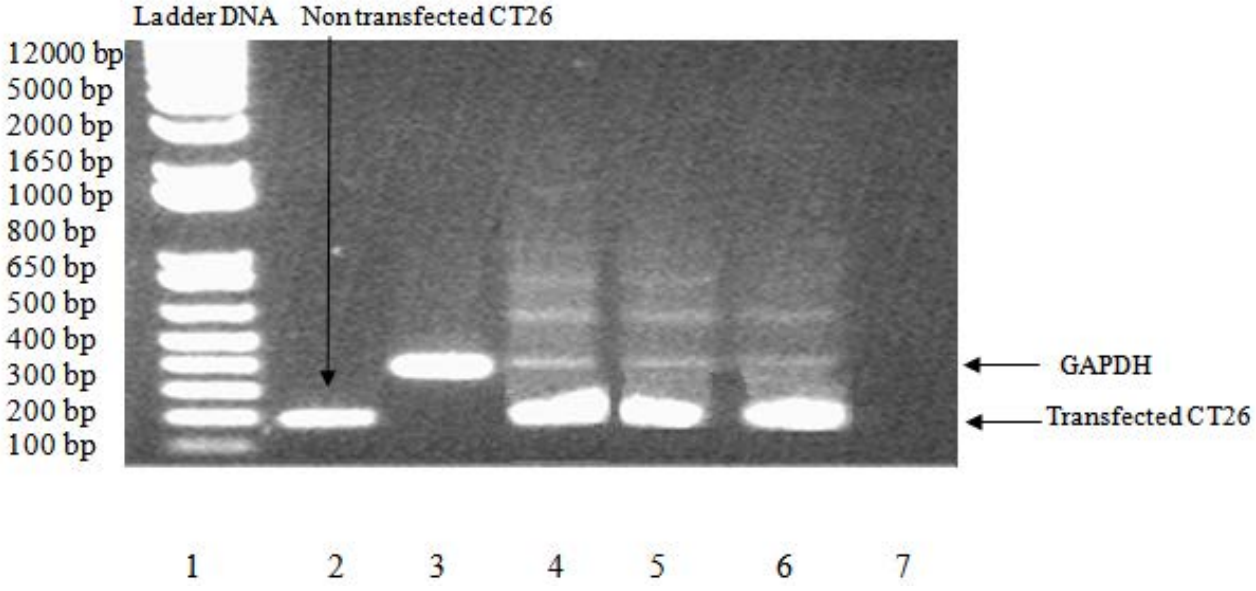

Figure 11. Expression of Ldcen-3 gene in transfected CT26 tumour cells as detected by RT-PCR

3.4.5. CTL activity in Balb/c mice by Immunisation with Ldcen-3construct Against Tumour Targets

Balb/c mice were immunised twice at a two week interval with Ldcen-3 construct coated on gold particles by gene gun. Mice were sacrificed two weeks following the 2nd immunisation and spleens were collected. Splenocytes were harvested and cultured in vitro for 5 days together with blasts cells pulsed with LPS and SLA2 (SLA may contain Ldcen-3 protein). On day 5, the splenocytes cells were used as effectors in a standard 4-hour ^{51}Cr-release cytotoxicity assay against CT26 tumour cells transfected with Ldcen-3 Figure 12. The results clearly show that immunisation of mice with Ldcen-3construct induce specific CTL activity against CT26 tumour cells expressing Ldcen-3. The in vitrorestimulation of CTLs by SLA2 loaded blast cells was crucial. It was shown that removing the in vitrorestimulation of the splenocytes prevented the generation of CTL activity in immunised mice and levels were comparable with that of naïve mouse splenocytes re-stimulated in vitro by blast cells loaded with SLA2. Maximum cytotoxity was observed even at the minimum effector to target (E:T) ratio of 6:1 suggesting the need for further testing with different effector to target ratios for unknown reasons.

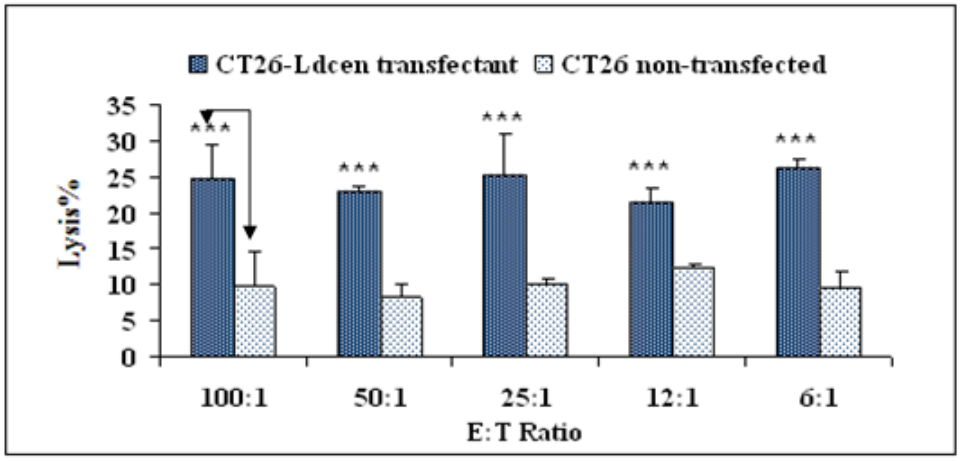

Figure 12. CTL activity of Balb/c mice immunised with 1µg pcDNA-Ldcen-3(-) by gene gun

4. Discussion

Immunisation with naked plasmid DNA represents a promising new approach in prevention and treatment of various diseases [18]. DNA vaccines offer a considerable number of advantages over other vaccines and are therefore an appealing approach to vaccination against Leishmaniasis. A number of studies have demonstrated encouraging results with DNA vaccines and have highlighted their potential in both treatment and protection against Leishmaniasis [19,20,21]. DNA vaccines are usually constructed from bacterial plasmids that are designed to express a gene of interest in the host cells to initiate antigen specific immune responses [22,23]. The plasmid DNA enters the cell and goes to the nucleus where it is transcribed to messenger RNA. The transcribed messenger RNA enters the cytoplasm and is translated on the ribosomes. The expressed antigen is presented to corresponding cells and generates a humoral and cell mediated immune response.

There is a homology in the gene sequence of Ldcen-3 between different species of Leishmania. Ldcen-3 appears to be a suitable candidate for a DNA vaccine, since Ldcen-3 is 100% homologous between L. donovani, L.mexicana and L. major [24]. Selvapandiyan and coworkers [14] have previously shown that immunisation with a live attenuated L. donovanicentrin 1 gene-deleted parasite (LdCen1) could induce significant protection against Leishmaniasis in animals. Balb/c mice immunised with LdCen1 (Leishmania mutant) demonstrated early clearance of virulent parasite challenge compared with mice immunised with killed parasites, which was associated with a significant increase of cytokine (IFN-γ, IL-2, and TNF) producing $CD4^+$ T cells. Immunised mice also showed increased IgG2a and NO production in macrophages. Balb/c mice immunised with LdCen1were cross-protected against L. braziliensis suggesting that LdCen1is a safe and effective vaccine candidate against visceral and mucocutaneousLeishmaniasis.

To transfect CT26 tumour cells with Ldcen-3 to be used as targets to assess CTL activities in Balb/c mice immunised with Ldcen-3 plasmid construct, it was decided to sub clone the Ldcen-3 from pCRT7/CT-TOPO into a known mammalian pcDNA 3.1 plasmid. Garmory and coworkers [25] have reported that pcDNA 3.1 is a suitable mammalian vector having the cytomegalovirus (CMV) promoter which is required for optimal expression in mammalian cells. Also, pcDNA3.1/hygro is a suitable vector for a DNA vaccine. pcDNA3, which is very similar to pcDNA3.1/Hygro, has been used in other studies as a back bone for DNA vaccines against Leishmaniasis [26,27]. Therefore, Ldcen-3 was sub cloned from pCRT7/CT-TOPO into pcDNA 3.1 to be used as a vaccine and also to be transfected into CT26 tumour cell to be used as target cells in CTL assays.

CT26 transfected with Leishmania centrin is expected to present centrin-3 antigen on their surface MHC I and would be a suitable target for CTL activity against Leishmania antigens. Stable transfectants expressing Leishmania antigens would provide a suitable alternative target to fresh DCs in cytotoxicity assays. Splenocytes from Balb/c mice immunised with pcDNA3.1-Ldcen-3 or pCRT7/CT-TOPO-Ldcen-3 induced a potent CTL response compared to the control group against either DC targets loaded with SLA2 or CT26 tumour cells expressing Ldcen-3. Tumour cells can act as professional APC that would specially generate CTL if they express a tumor peptide-MHC class I complex and co-stimulatory molecules [28,29]. The immunogenicity of Ldcen-3cDNA cloned in the pCRT7/CT-TOPO plasmid and pcDNA3.1 was determined via DNA vaccination in a Balb/c mouse model in vivo. Mice immunised with Ldcen-3 in pCRT7/CT-TOPOor in pcDNA3.1 (-) were significantly protected against challenge with live parasites; the known immunogenicity gp63 gene was used as a positive control. A dominant Th1 response was shown to have been correlated with protection in several animal models for Leishmania infection. Immunisation of Balb/c mice with a plasmid DNA vaccine containing gp63 gene from L. major, induced a dominant Th1 response that was protective against challenges with live parasites in vivo [17,30]. Susceptibility of Balb/c mice to Leishmania major infection was correlated to an inability to generate a Th1 response which could be restored by administration of IL-12 [31,32]. This Th response would aid the development of $CD8^+$ CTLs capable of killing cells expressing appropriate antigen.

DNA vaccines produce potent CD8 CTL responses in mice against antigens from parasites and tumours. The construction of DNA vaccine-encoded antigens able to produce a CTL response includes whole protein, truncated protein and fusion with another protein [33,34]. Conry et al. [28] and Jacobsen et al. [35] have found that if the

tumour cells are tranfected with plasmid DNA containing a tumour antigen gene then a specific CTL may be generated. In this work, CT26 tumour cells transfected with pcDNA3-Ldcen-3 were shown to be susceptible target cells to CTLs derived from Balb/c mice immunised with pcDNA3-Ldcen-3by gene gun. Ali et al. [17] have demonstrated a potent CTL activity in cultured splenocytes from Balb/c mice immunised L. mexicana gp63 DNA plasmid using I.M. injection and gene gun immunisation with against CT26 tumour cells transfected with pcDNA-gp63. Qin et al. [36] have shown a method of DNA immunisation using a prime-boost immunisation strategy (two different vaccines, each encoding the same antigen, given several weeks apart); better protection was obtained by gene gun immunisation. In addition, Gurunathan et al. [37] have reported the presence of long term antigen-specific Th1 activity in mice immunised with a DNA vaccine containing a gene that coded for a Leishmania antigen. Rodriguez-Cortes et al. [1] found that a multivalent DNA vaccine, encoding TRYP which is a key enzyme of the trypanothione dependent metabolism for removal of oxidative stress in Leishmania, LACK and gp63, did not protect dogs against L. infantum experimental challenge, inspite of the hypothesis that an effective immune response was more likely to be generated following exposure to more than one antigen. Alternatively, Carter et al. [38] established that Balb/c mice immunised intramuscularly by parasite enzyme gamma glutamyl cysteine synthetase DNA vaccine protected them against L. donovani.

In conclusion, this study had shown for the first time that Balb/c mice immunised with pcDNA 3.1-Ldcen-3 or pCRT7/CT-TOPOLdcen-3constructs by gene gun induced potent protection against challenge with L. mexicana which was also correlated with CTL activity.

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