Vaccine 31 (2013) 4682–4688

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Carrier protein influences immunodominance of a known epitope: Implication in peptide vaccine design

Moumita Ghosh [a], Ashish K. Solanki [b], Koushik Roy [a], Reema R. Dhoke [b], Ashish [b], Syamal Roy [a,*]

[a] Division of Infectious diseases and Immunology, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata 700032, India
[b] CSIR-Institute of Microbial Technology, Chandigarh, India

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 10 June 2013
Accepted 14 June 2013
Available online 5 August 2013

Keywords:
Immunodominance
Antigen processing
Antigen structure
Endoproteolysis

ABSTRACT

We investigated how the processing of a given antigen by antigen presenting cells (APC) is dictated by the conformation of the antigen and how this governs the immunodominance hierarchy. To address the question, a known immunodominant sequence of bacteriophage lambda repressor N-terminal sequence 12–26 [$\lambda R_{(12-26)}$] was engineered at the N and C termini of a heterologous leishmanial protein, Kinetoplastid membrane protein-11 (KMP-11); the resulting proteins were defined as N-KMP-11 and C-KMP-11 respectively. The presence of $\lambda R_{(12-26)}$ in N-KMP-11 and C-KMP-11 was established by western blot analysis with antibody to $\lambda R_{(12-26)}$ peptide. N-KMP-11 but not C-KMP-11 could stimulate the anti $\lambda R_{(12-26)}$ T-cell clonal population very efficiently in the presence of APCs. Priming of BALB/c mice with N-KMP-11 or C-KMP-11 generated similar levels of anti-KMP-11 IgG, but anti-$\lambda R_{(12-26)}$ specific IgG was observed only upon priming with N-KMP-11. Interestingly, uptake of both N-KMP-11 and C-KMP-11 by APCs was similar but catabolism of N-KMP-11 but not C-KMP-11 was biphasic and fast at the initial time point. Kratky plots of small angle X-ray scattering showed that while N-KMP-11 adopts flexible Gaussian type of topology, C-KMP-11 prefers Globular nature. To show that KMP-11 is not unique as a carrier protein, an epitope (SPITBTNLBTMBK) of *Plasmodium yoelii* (PY) apical membrane protein 1[AMA-1 $_{(136-148)}$], is placed at the C and N terminals of a dominant T-cell epitope of ovalbumin protein $OVA_{(323-339)}$ and the resulting peptides are defined as PY-OVA and OVA-PY respectively. Interestingly, only OVA-PY could stimulate anti-OVA T-cells and produce IgG response upon priming of BALB/c mice with it. Thus for rational design of peptide vaccine it is important to place the dominant epitope appropriately in the context of the carrier protein.

1. Introduction

Peptide vaccines are gaining importance with time because of their inherent advantages [1–5]. Until now not a single peptide vaccine has crossed phase III trial [6]. Unfortunately the progress so far in developing successful synthetic vaccines has been disappointing, reflecting the limitations in our understanding [7]. Most of the attempts to develop synthetic peptide vaccines are based on the assumption that linear peptides possess similar conformation as observed in the pathogens or in the protein from which they has been derived [8]. Peptides coupled to a protein carrier have shown to induce immunogenic response [9]. But it is yet not clear whether the immunodominant pattern of these antigenic peptides will be preserved in a new protein context. A significant body of literature suggest that peptides apparently play a minor role in immunodominance [10,11] but there are alternative views too [12–20]. Thus lack of success in developing synthetic peptide vaccine calls for a fresh look.

There are two views on epitope generation from the repertoire of dominant sequences present in a given sequence [21]. These are 'MHC-guided epitope generation' and 'protease dependent epitope generation' and they may or may not be mutually exclusive [21,22]. In the 'MHC guided event', the MHC molecule scans large antigenic fragments and binds to the determinant with highest affinity, thus protecting the epitope from further proteolytic cleavages. There are evidences to support such notion, e.g. lysozyme[23] and fibrinogen [24] bind to MHC II molecule before being processed. In contrast, protease-dependent epitope generations are not available for MHC II binding until the antigen's structure is unlocked by one or more

Abbreviations: APC, antigen presenting cell; KMP-11, kinetoplastid membrane protein-11; $\lambda R_{(12-26)}$, immunodominant sequence of bacteriophage lambda repressor N-terminal sequence; N-KMP-11, $\lambda R_{(12-26)}$ engineered at the N termini of KMP-11; C-KMP-11, $\lambda R_{(12-26)}$ engineered at the C termini of KMP-11; $OVA_{(323-339)}$, dominant T-cell epitope of ovalbumin protein; AMA-1, *plasmodium yoelii* apical membrane protein 1; PY, an epitope of AMA-$1_{(136-148)}$.

* Corresponding author. Tel.: +91 33 2499 0395; fax: +91 33 2472 5197.
E-mail addresses: drsyamalroy@yahoo.com, sroy@iicb.res.in (S. Roy).

0264-410X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2013.06.110

endoproteolytic cleavages [22]. Earlier it was shown that minimal essential structure of an epitope is required for T-cell binding [25]. But a general relationship between the structure of a protein antigen and immunodomninace of a given epitope as a function of MHC polymorphism is yet to be established. We endeavored to learn the 'complex rules' by which protein antigens are subjected to be 'scrutinized' by the immune system.

In this investigation two defined antigens have been used as probes. In the first case a known immunodominant (12–26) peptide sequence of bacteriophage lambda repressor (LEDARRLKAIYEKKK) protein, described as $\lambda R_{(12-26)}$, was grafted into the heterologous kinetoplastid membrane protein 11 (KMP-11) of *Leishmania* parasite at different locations by recombinant technology. In the other case an important epitope of *Plasmodium yoelii* apical membrane protein 1(AMA1) was placed in tandem at the C and N terminals of a dominant T-cell epitope of ovalbumin protein ($OVA_{323-339}$) by peptide synthesis. Both the antigens were used to study the immunodominance of the known epitopes and the outcome of such study is quite striking. Our study indicates that the dominance of an epitope is not intrinsic and the environment around the epitope may play a decisive role.

2. Materials and methods

2.1. Ethics statement

Use of mice and rabbit was approved by the Institutional Animal Ethics Committee of Indian Institute of Chemical Biology, India. All animal experimentations were performed according to the National Regulatory Guidelines issued by CPSEA (Committee for the Purpose of Supervision of Experiments on Animals), Ministry of Environment and Forest, Govt. of India.

2.2. Cell line

T cell hybridomas 9H3.5 (I-A^d restricted) and 10I (I-E^k restricted) specific for $\lambda R_{(12-26)}$ [26] was a gift from Prof. M L Gefter (Massachusetts Institute of Technology, Cambridge, MA) and 3DO54.8 (A^d restricted), specific for $OVA_{323-339}$ [27] was a gift from Prof. Peter Walden (Humbolt University, Charité, Berlin, Germany).

2.3. Recombinant purified proteins

Kmp-11 constructs were designed and purified as described earlier [28]. The details are described in the supplementary section. Hereafter, the N-terminal and C-terminal cloned epitope tags $\lambda R_{(12-26)}$ are denoted as N-KMP-11 and C-KMP-11 respectively.

2.4. Isolation of peritoneal exudate cells (PEC)

BALB/c and CBA/Caj mice were intraperitoneally injected with 3 mL of 4% starch. After 48 h PEC were isolated and plated on tissue culture petri dishes in complete RPMI medium for 48 h at 37 °C in presence of a 5% CO_2 atmosphere. Non-adherent cells were removed thereafter by gentle washing with serum free medium. The adherent PECs is defined as antigen presenting cells (APC) for convenience henceforth.

2.5. Antigen presentation assay

APCs were co-cultured with 10^5 T-cell hybridomas in presence and absence of antigen. After 18 h, the supernatant was collected and resulting IL-2 production was measured by ELISA [29].

2.6. T-cell proliferation and antibody response

BALB/c mice were immunized in the foot pad with 80 μg of antigen emulsified with CFA [30]. After seven days, draining lymph nodes were harvested, pooled and cultured in RPMI medium supplemented with 10% FCS. Lymph node cells were plated (5×10^5cells/well) into 96 well microtiter plates in triplicate with Concanavalin A, $\lambda R_{(12-26)}$ peptide, N-KMP-11 or C-KMP-11. After 72 h T-cell proliferation was measured by MTT [31].

BALB/c mice were immunized as above and were kept for 21 days. Secondary immunization was followed and the blood was collected on the 7th day of secondary immunization. The antibody titer against $\lambda R_{(12-26)}$ peptide and the KMP-11 protein were measured by ELISA. The $\lambda R_{(12-26)}$ peptide was coupled with BSA using EDAC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide] as described [32].

2.7. Antigen uptake and catabolism

The recombinant proteins were radioiodinated with 5 μL (500 μCi) of $Na^{125}I$(17 mCi/μg) following the protocol previously reported [33]. N-KMP-11 and C-KMP-11 protein, 5 μCi/μg each, were added to 5×10^5cells/well of APCs plated in 48 well tissue culture plate as required. The APCs were washed thoroughly and 1% Triton X 100 was added. Radioactive counts were made on solubilized APC extract [33]. The catabolism was studied following the protocol as reported previously [34]. Percent catabolism was expressed as $100-[(c.p.m_{total} - c.p.m_{TCA\ soluble})/c.p.m_{total} \times 100]$.

2.8. Peptide synthesis

We have designed peptides by inserting the $Ovalbumin_{(323-339)}$ peptide (OVA) (ISQAVHAAHAEINEAGR) at N-terminal or C-terminal end of $AMA\text{-}1_{(136-148)}$ peptide (SPITBTNLBTMBK) from *Plasmodium yoelii* (PY) respectively. The PY sequence was synthesized in relation to OVA sequence as follows: OVA-PY (ISQAVHAAHAEINEAGRKKSPITBTNLBTMBK) and PY-OVA (SPITBTNLBTMBKKKISQAVHAAHAEINEAGR). All the peptides were synthesized on Rink Amide MBHA resin using standard solid phase Fmoc chemistry [35] and involving a capping step with 5% acetic anhydride and 5% lutidine in DMF after each coupling using PS3 peptide synthesizer (Protein Technologies Inc, USA). Fmoc-amino acids were activated with HBTU in presence of HOBt and DIEA. Peptides were cleaved from the resin and side-chain protecting groups were removed by incubating with 94% TFA, 2.5% EDT, 1.5% thioanisole, 1.5% water and 0.5% TIS for 3 h at room temperature, and ice-cold diethyl ether was added for precipitation. Peptides were then purified by HPLC (Waters, USA) on a reverse phase μbondapak C-18 column using 0–80% acetonitrile in 0.01% TFA and the molecular weights were determined by MALDI-TOF/TOF analyzer (Applied Biosystem, USA).

3. Results

3.1. Characterization of recombinant proteins

The three recombinant proteins, KMP-11, N-KMP-11 and C-KMP-11, were identified in western blot with anti-KMP-11 antibody. The presence of the epitope $\lambda R_{(12-26)}$, in N-KMP-11 and C-KMP-11 was confirmed by western blot with anti-$\lambda R_{(12-26)}$ antibody (Fig. 1A).

3.2. Functional analysis of immunodominance

The ability of C-KMP-11 and N-KMP-11 to stimulate T-cell hybridomas 9H3.5 and 10I in association with appropriate APCs