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Identification and characterization of Th cell epitopes in MrkD adhesin of *Klebsiella pneumoniae*

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ABSTRACT

In this study, we identified the Th epitopes in MrkD of *Klebsiella pneumoniae*, an excellent vaccine candidate antigen. By using the RANKPEP prediction algorithm, we have identified and characterized three Th epitopes within the MrkD antigen, which can be recognized by CD4+ T cells from BALB/c (H-2^d) mice. They were $M_{221\text{-}235}$, $M_{175\text{-}189}$, and $M_{264\text{-}278}$. These epitopes have important value for studying the immune response of *K. pneumoniae* infection and for designing effective vaccine against *K. pneumoniae*.

1. Introduction

Klebsiella pneumoniae is an important cause of respiratory infections and is responsible for significant morbidity and mortality in compromised individuals [1]. Even though antibiotics are still the most effective treatment for *K. pneumoniae* infection, multi-antibiotic resistance becomes a more pronounced problem. Thus, it is of great interest to find alternative ways to control the *K. pneumoniae* infection. Vaccination has become one of the most promising approaches against *K. pneumoniae* infection.

Currently, most of vaccines against *K. pneumoniae* were reported to use native components from *K. pneumoniae* such as fimbriae and capsular polysaccharide and LPS [2–5]. In general, these treatment are based on a natural form of the pathogen. In response to *K. pneumoniae* infection, the host triggers vigorous humoral and cellular immune response. CD3+ CD4+ cells increase in the *K. pneumoniae*+ pulmonary lamina propria and T cell infiltration is correlated with grades of pneumonia, activity and density of bacterial colonization. In spite of robust immune responses, the infection is still persistent and even for life long, suggesting that *K. pneumoniae* can evade both adaptive and innate immune responses and the immune responses triggered by *K. pneumoniae* in nature were not optimal to eliminate this pathogen. The immune responses evoked by natural infection or subunit vaccine immunization are not favorable. The immune responses should be improved and modified [6]. Therefore, we assume that the modified immunotherapy is a feasible treatment which may be effective to combat against *K. pneumoniae*. This purpose can probably be achieved by artificially promoting qualitatively or quantitatively immunity different from natural infection.

Epitope-based vaccines represent a modified immunotherapeutic approach which is based on the observation that in some instances. The potential advantages of the epitope-based approach include a specific immune response, increased safety, increased potency and breadth of rationally engineered epitopes and focusing on immune responses elicited by conserved epitopes [7]. Accordingly, rational choices are made to isolate the components desired for the responses. The premise of these efforts is the identification of the appropriate epitopes [6].

The mechanisms of protection against *K. pneumoniae* infection remain unclear, but there are growing evidences to support an important role of CD4+ T cell in the protection against *K. pneumoniae* [8]. The requirement of major histocompatibility complex (MHC) class II for protection in mice implies an important role of CD4+ T cell [8,9]. These findings, along with the important role of CD4+ T cell in supporting both B-cell and CD8+ T cell function, suggest that a successful immunotherapeutic or immunoprophylactic strategy against *K. pneumoniae* should include CD4+ Th cell epitopes.

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It was demonstrated that MrkD adhesin mediated *K. pneumoniae* to adhere human respiratory tissue [10,11]. The MrkD adhesin has been considered as an excellent vaccine candidate antigen and found to be protective in mice [12], but the immune mechanism that provide protective immunity are not yet fully understood. Knowledge of epitopes in MrkD and their correlation with protection is limited primarily to the identification of B cell epitopes [12]. However, there is no information available about T cell epitopes from MrkD. We think that characterization of the CD4+ T cell epitopes of MrkD would also be vital in understanding the immune responses of *K. pneumoniae* infection and developing new immunoprophylactic and therapeutic strategy against *K. pneumoniae*. So, the present study aimed to identify the Th epitopes of MrkD.

2. Results

2.1. Prediction of potential MrkD Th epitopes and synthetic peptides

In order to study the immune response of BALB/c mice, the potential binding motifs for I-Ad and I-Ed in amino acid sequence of MrkD were scanned by the RANKPEP software. The I-Ad and I-Ed restricted epitopes were predicted, respectively. We selected five putative I-Ad restricted and one I-Ed restricted epitopes with higher scores (Table 1). The selected peptides were synthesized and the purities of these peptides were all ≥85% analyzed by high-pressure liquid chromatography.

2.2. CD4+ T cell proliferation responses to MrkD-derived peptides

To define the epitopes within MrkD that are recognized by CD4+ T cells, we isolated CD4 positive cells from BALB/c mice immunized with rMrkD and cultured those cells along with Co^{60} irradiated syngeneic splenic APC and synthetic peptides, as described in Section 4. In preliminary experiments, we determined the optimal concentration of synthetic peptides at 1 μg/ml and rMrkD at 20 μg/ml. As shown in cell proliferation, CD4+ T cells isolated from rMrkD vaccinated mice, but not from PBS treated mice responded to peptides $M_{221\text{-}235}$, $M_{175\text{-}189}$, and $M_{264\text{-}278}$, and rMrkD (SI > 2,Fig. 1A). The irradiation treated-APCs could not be stimulated by ConA, which confirm that ^{3}H-TdR incorporation is not due to the proliferation of APCs. CD4+ T cells without APCs could not proliferate either (SI < 2, Fig. 1B). So we infer CD4+ T cells proliferation is the response to peptides, not non-specific response such as mitogen stimulation. Furthermore, these responses were antigen-specific, as the cells were primed by rMrkD but not by PBS (Fig. 1A). These results indicate that the three peptides ($M_{221\text{-}235}$, $M_{175\text{-}189}$, and $M_{264\text{-}278}$) contain the Th cell epitopes for BALB/c mice.

2.3. MHC restriction of the synthetic peptide-specific lymphocyte proliferation

CD4+ T cells recognize peptides presented by class II MHC molecules on the surface of APCs. The interaction between T cells and the peptide-MHC class II complex can be blocked by the mAbs against MHC class II molecule. In BALB/c mice, MHC class II molecule includes two types: I-Ad and I-Ed. Specificity of restriction was determined by the inclusion of specific anti-MHC class II mAb in the T-cell proliferation assay. As Fig. 2 shows, I-Ad-specific mAb was able to inhibit the lymphocyte proliferation to $M_{221\text{–}235}$ and $M_{175\text{-}189}$, while anti-I-Ed and anti-MHC I mAbs had no such effects. MAb specific for I-Ed inhibited the T-cell responses to $M_{264\text{-}278}$. Therefore, these results indicate T cell can recognize $M_{221\text{-}235}$, $M_{175\text{-}189}$, and $M_{264\text{–}278}$ in the MHC class II molecule context and further confirm that the three peptides are CD4+ T cell epitopes.

Table 1
Results of prediction of potential Th epitope.

Peptide	MHC restriction	Amino acid sequence	Rankpep scores
$M_{169\text{–}183}$	I-Ad	PILETYLSANAITVV	20.65%
$M_{221\text{–}235}$	I-Ad	IELQCSGALSETGYA	19.25%
$M_{175\text{–}189}$	I-Ad	LSANAITVVSPSCSV	18.58%
$M_{19\text{–}33}$	I-Ad	TSSWAVCTRLSSPTV	18.39%
$M_{281\text{–}295}$	I-Ad	QFNKKYTVGRLNNQE	16.00%
$M_{264\text{–}278}$	I-Ed	GMAKGVGIQVLKDGS	20.99%

Note: $M_{169\text{–}183}$ represents the amino acid 169–183 of MrkD, the same to others.

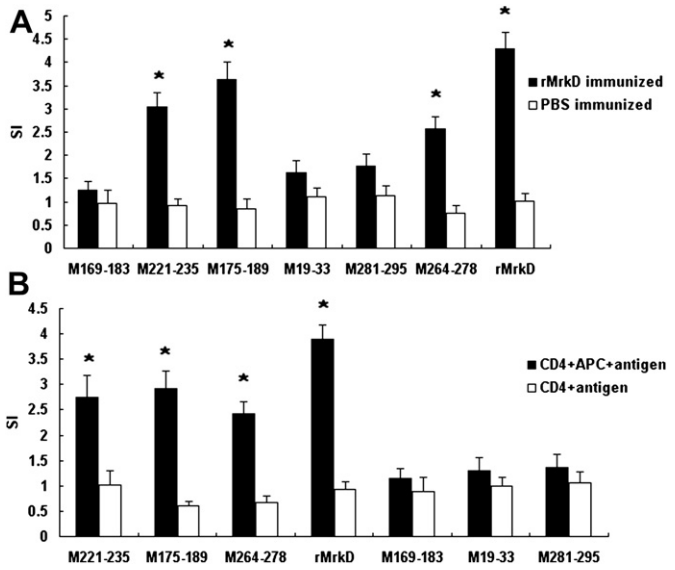

Fig. 1. Peptides specific CD4+ T cell proliferation in BALB/c mice immunized with rMrkD. CD4+ T cells primed with rMrkD were tested in a proliferation assay for responses to synthetic peptides (1 μg/ml), rMrkD (20 μg/ml) and control medium. Response to the antigen is expressed as the mean SI of three independent experiments ± S.D. SI ≥ 2 is considered as positive. (A) CD4+ T cells isolated from mice treated with PBS served as controls to determine if the responses were rMrkD-specific. (B) CD4+ T cell did not response to peptides and rMrkD in absent of APC. (*) SI is higher than 2 at the 95% confidence level.

2.4. Cytokine profile of lymphocyte induced by MrkD derived peptides

To determine the subset of CD4+ T cells induced by each MrkD derived-peptide, we analyzed the cytokine profile of lymphocytes in response to the peptides. As shown in Fig. 3, CD4+ T cells isolated from mice vaccinated with rMrkD specifically produced significant

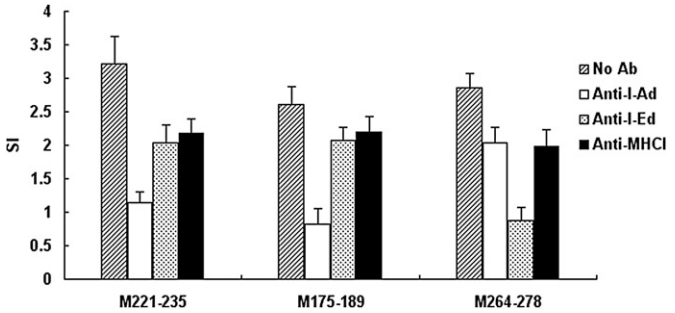

Fig. 2. Inhibition of the lymphocyte proliferation by MHC class II-specific mAb. Splenic lymphocytes from mice primed with rMrkD were stimulated with peptides (1 μg/ml) in the presence of mAbs specific for I-Ad, I-Ed, or MHC class I molecules. Cultures incubated with peptides without mAbs served as controls. The proliferation of the cells was quantified in triplicate by ^{3}H-TdR incorporation.