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A single epitope of Epstein-Barr Virus stimulate IgG production in mice

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ABSTRACT

Background: Epstein-Barr virus (EBV) is closely associated with the high incidence of nasopharyngeal carcinoma in worldwide. Vaccination is one strategy with the potential to prevent the occurrence of EBV-associated cancers, but a suitable vaccine is yet to be licensed. Much vaccine development research focuses on the GP350/220 protein of EBV as it contains an immunogenic epitope at residues 147–165, which efficiently stimulates IgG production *in vitro*. We examined the ability of this epitope (EBVepitope) to induce IgG production in mice.
Methods: The antibody binding pattern of the epitope was analyzed using bioinformatics tools. The IgG production in mice were examined by FACS Calibur™ Flow cytometer.
Results: The epitope bound the 72A1 monoclonal antibody at the same site as GP350/220 protein, indicating that the epitope should stimulate B cells to produce antibody. Moreover, *in vivo* administration of EBVepitope successfully induced IgG expression from B cells, compared with controls. Further investigation indicated that the relative number of B cells expressing IgE in EBVepitope-treated mice was lower than controls.
Conclusions: Our data suggest that this EBV GP350 epitope is able to induce IgG expression *in vivo* without causing allergic reactions, and represents a potential EBV vaccine candidate.

1. Background

Epstein-Barr Virus (EBV) has been implicated in the development of various malignancies [1] such as nasopharyngeal carcinoma (NPC) [2,3] lymphoepithelioma [4], gastric carcinoma [5], and progression of cervical cancer [6]. This virus is strongly associated with the high incidence of NPC in the worldwide [7] and therefore, preventative therapeutics are urgently needed. Vaccination is one way to prevent EBV infection or EBV caused-disease. The vaccine also could be used as therapeutic agent to prevent EBV spread from cell-to-cell within a host and thus reduce the incidence of EBV-driven cancers. However, the vaccine of the EBV has not been licensed.

Identification of an antigenic viral protein that binds to a host receptor is an important goal in EBV vaccine development. GP350/220 protein of EBV is known to bind to CD21/CR2 on the host cell surface, facilitating the virus infection [8]. Monoclonal antibodies (mAbs) against this protein have been shown to neutralize the EBV [8]. Administration of mAbs against the GP350/220 protein have also been shown to prevent the development of EBV-positive tumors in animal models [9]. Therefore, this epitope shows promise as a candidate EBV vaccine against NPC.

In our previous study, we found that GP350/220 protein contains an epitope that can stimulate *in vitro* antibody production [10]. The epitope between amino acid residues 147–165 of GP350/220 protein are conserved in several EBV strains [11]. Thus, epitope-based vaccine development using this epitope is very promising, with the potential to be effective across multiple strains of EBV. Herein, we modeled the epitope structure, its binding activity to monoclonal antibody against EBV, 72A1 mAb, and also tested the ability of candidate vaccine epitope to stimulate IgG production in experimental animals.

2. Methods

2.1. Modeling the tertiary structure of peptides and proteins

The epitope peptide of EBV was identified previously [11], and designated EBVepitope for the purposes of this study. The tertiary (3D) structure of EBVepitope was then modeled using the PEPstrMOD server [12]. Meanwhile, the antibody structure was also modeled according to the mAb 72A1 heavy- and light-chain variable region sequences [13] by using the antibody variable region of homology modeling server, RosettaAntibody [14]. The results of modeling were then visualized with Discovery Studio, BIOVIA [15].

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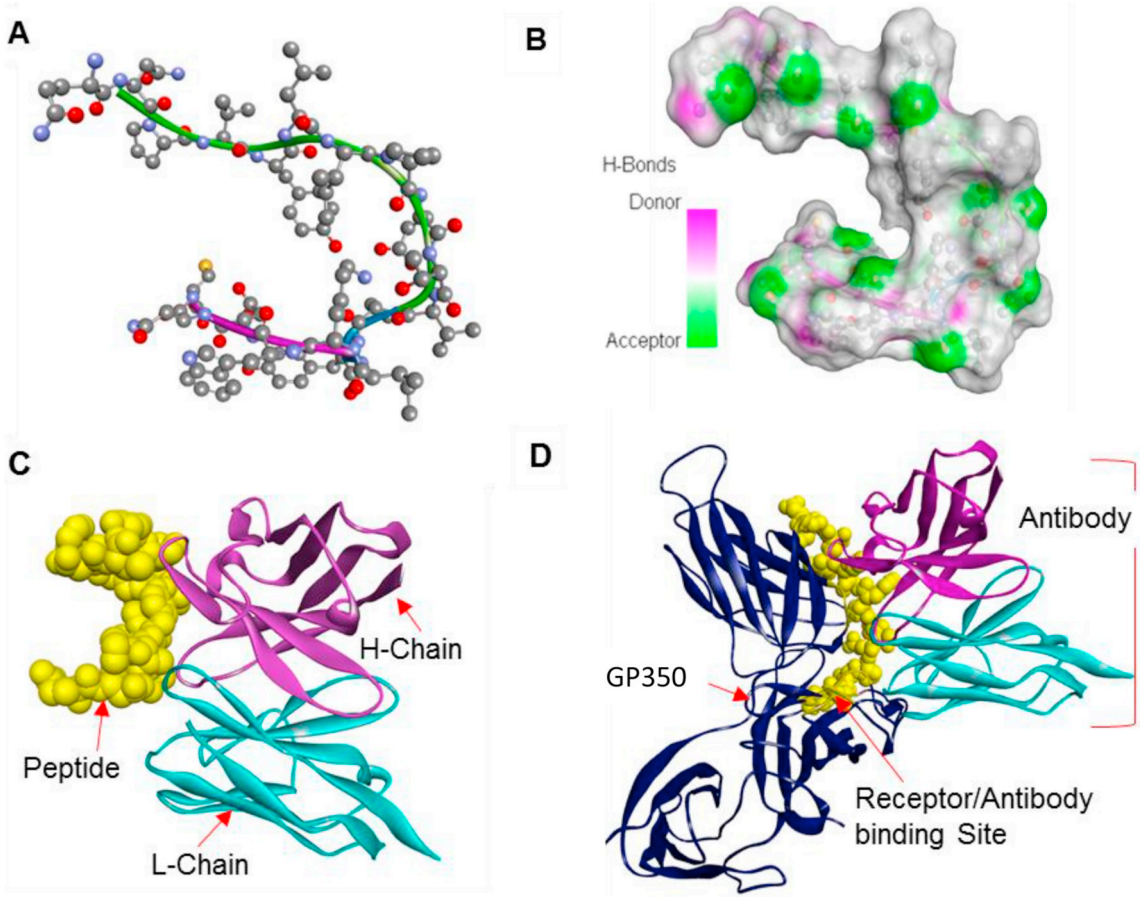


Fig. 1. The tertiary structure of EBVepitope (A) showed a coil structure with many hydrogen donors/acceptors (B). The epitope could bind 72A1 mAb in both the heavy- (purple) and light-chain (cyan) regions (C), which was similar to the binding pattern between the intact GP350/220 protein and 72A1 mAb (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Protein interaction analysis

The binding pattern of EBVepitope with the 72A1 mAb was done using the PatchDock server [16]. Binding patterns of the antibody-epitope complex were then compared with the binding pattern of GP350/220 to the mAb. The 3D structure of the GP350/220 protein was taken from the Protein Data Bank with accession code 2H6O [17]. The interaction of peptide amino acids with the antibody was analyzed by LigPlot + program [18] and tabulated (Fig. 2A).

2.3. Peptide preparation and animal treatment

EBVepitope [9] was synthesized by Genescript and then used as an antigen to induce antibody production in BALB/c mice. In this study, we used twelve 3-week-old male mice with body weight ± 10 g, divided into four groups. Each group of mice was subcutaneously injected with 50 μg of peptide (~22 μL) mixed with 200 μL of adjuvant (pept +), 50 μg (~22 μL) peptide (pept), 40 μg (~20 μL) lipopolysaccharide (LPS) and ~20 μL phosphate buffered saline (PBS) as a control group. Two injections were given, with the second injection being given on day 7 as a booster. Complete Freund's Adjuvant was used for the first injection, followed by Incomplete Freund's Adjuvant in the booster inoculation on day 7. The experimental animals were maintained in cages with unrestricted access to food and water.

2.4. Lymphocyte cell isolation and immunostaining

The treated mice were sacrificed by neck dislocation; spleens were then collected and washed with 1 mL of sterile PBS. Next, spleens were crushed with the base of a syringe, filtered through sterile gauze and the homogenates were suspended in 5 mL of PBS. The suspension was centrifuged at 2500 rpm at 4 °C for 5 min. The supernatant was then discarded, and the pellet was resuspended in 1 mL of PBS. Fifty microliters of the suspension was taken and put in a 1.5-mL sterile microtube containing 300 μL of PBS. The microtube was centrifuged at 2500 rpm at 4 °C for 5 min. The supernatant was then removed, and the pellet was resuspended in the residual PBS and immunostained for flow cytometric analysis. Forty microliters of fluorescein isothiocyanate (FITC)-conjugated anti-mouse B220 (clone: RA3-6A2; BioLegend, San Diego, CA), PE-conjugated anti-mouse IgE (clone: RME-1; BioLegend) and PE-Cy7-conjugated anti-mouse IgG (clone: Poly 4053; Biolegend) was added to each sample.

2.5. Cell counting and data analysis

The stained cells were diluted in 400 μL of PBS and put in a flow cytometer cuvette for analysis on a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA) to calculate the relative numbers of B cells expressing IgG and IgE. The data were analyzed by the normality test and homogeneity test of variance, followed by a one-way ANOVA.

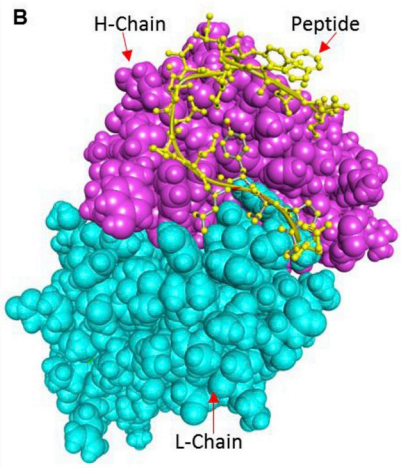
3. Results

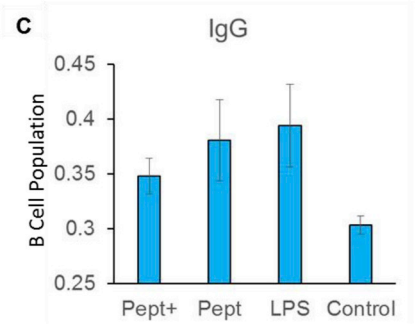
The modeling analysis of EBVepitope showed that the epitope structure had a coil shape and was therefore very similar to its native structure in the intact protein, GP350/220 (Fig. 1A). This structure of EBVpeptide is stable as it is formed by many hydrogen bonds (Fig. 1B). Due to the stability of the EBVepitope structure, it successfully bound to

A

Interaction of the Peptide with Antibody

Peptide	H-Chain	Peptide	L-Chain
Pro8	Tyr98	Leu6	Tyr32
	Tyr100	Tyr5	Tyr94
Leu6	Tyr100	Val4	Ser93
Ile7	Tyr100		Tyr94
	Trp95		Tyr96
Tyr5	Trp95		Tyr92
	Tyr50	Asn2	Ser93
	Ser52		Tyr94
Asp17*	Thr56*	Pro3	Ser93
Tyr13	Ser55		Tyr94
Pro12	Ser55		
	Ser54		
	Gly53		
	Ser52		
Val11	Ser52		
Cys19	Leu57		
	Ser52		
Glu9*	Asn97*		





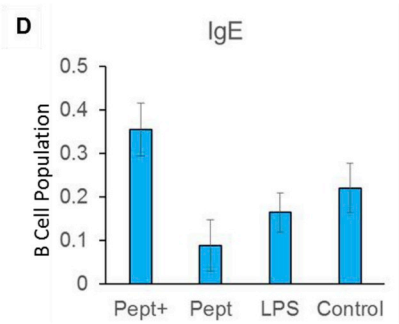


Fig. 2. The EBVpeptide interacted with 72A1 mAb in both the heavy and light chains (A and B), and was able to induce IgG production in B cells compared with the control (C). Administration of EBVepitope alone was sufficient to stimulate IgG without triggering IgE production (D). Error bar of Histogram indicated Standard deviation of within the group. The ANOVA test suggested that among group of the treatment was significantly different; with P value is 0.0168 for IgG expressed B cell population, and P value is 0.0022 for IgE expressed B cell population. *indicated by the amino acid forming the hydrogen bond; Pept+, EBVepitope mixed with Adjuvant; Pept, EBVepitope alone; LPS, lipopolysaccharide.

the anti-GP350/220 mAb, 72A1. The peptide could bind in both the heavy- and light-chain variable regions of the 72A1 mAb. We then compared this with the binding pattern of the 72A1 mAb with EBVepitope and GP350/220. The results of this analysis indicated that EBVepitope and GP350/220 bound 72A1 at the similar site. The similarity in binding pattern between the peptide and the intact protein indicates that the peptide should be able to stimulate B cells to produce antibodies in a manner similar to the whole protein.

To further elucidate the molecular interaction between the 72A1 mAb and EBVepitope, we used LigPlot + software. The analysis results of these interactions indicated that the majority of peptides interacted with the heavy chain rather than the light chain of the variable region of 72A1 mAb (Fig. 2A and B). Analysis of multiple interactions showed that the bonds between molecules were vigorous and stable, illustrating that EBVepitope can bind the antibody in a stable manner, so it is likely that the peptide is able to bind the native cell-bound receptor and stimulate B cells to produce antibodies.

We next tested the ability of EBVepitope in inducing the production of antibodies *in vivo* using a mouse model. Our results indicated that the peptide was able to induce the production of IgG in mice. The number of B cells ($B220^+$ cells) that expressed IgG increased in mice injected with either peptide alone (38.08%) or peptide plus adjuvant (pept+; 34.81%), compared with the control (30.34%) (Fig. 2C). Injection of LPS also successfully induced IgG production (39.4%).

4. Discussion

Our data confirmed that administration of this specific peptide sequence was able to increase the production of IgG antibodies in mice, compared with controls. Moreover, EBVepitope alone was capable of inducing IgG expression better than EBVepitope plus adjuvant. This may be a result of an imbalance in the ratio of EBVepitope to adjuvant in the injected suspension. Too much adjuvant could result in depot effects, or the antigen could be swamped by the adjuvant [19] or by serum IgM. This would make it difficult for B cells to directly access the peptide, thus limiting the induction of IgG.

Vaccination can induce CD203c expression in basophils, which may stimulate IgE production [20]. Such an increase in IgE levels is undesirable and should be avoided to prevent potential vaccination side effects. However, we found that the relative number of B cells expressing IgE in EBVepitope-treated mice was lower than that observed in controls (Fig. 2D). Furthermore, the relative numbers of IgE-expressing B cells in the pept + group was higher compared with the control. These data indicate that administration of adjuvant increased the risk of allergic reaction following vaccination, as the adjuvant likely contained a substance that was able to increase IgE production [21].

Based on the data suggesting the epitope has potential for developing the vaccine candidate for control proliferation of EBV. Vaccine is one of important step to prevent infection of EBV and also cancer epidemiology, since the EBV infected 90% of world population [22] and linked to the multiple cancers. Even the prevalence might related to the socio-economic of the population. In most cases, EBV-infected individu has no symptom, but if the virus-host balance is disturbed, could be associated with several lymphomas. In Argentina, EBV infection is mostly subclinical and have seropositive by 3 years old [23]. Seroprevalence of EBV also increase in US ranging from 54.1% for 6–8 year olds and 82.9% for 18–19 year olds in 2010 [24]. Therefore, vaccination for the childhood is very important to prevent the EBV caused diseases. However, even though several vaccine candidate has been examine in the clinical trial but none is licensed [25].

5. Conclusion

Our chosen epitope on the GP350/220 protein of EBV has a stable coil structure, which is very similar to its native structure in the intact protein. Therefore, this epitope remains able to recognize the 72A1

mAb against GP350/220 protein. Furthermore, this stability of structure retains the immunogenicity of the epitope *in vivo*, facilitating the induction of IgG in mice. In the absence of adjuvant, IgG is increased, while undesirable IgE remains low. Our findings suggest that this peptide has the potential to be developed as a safe vaccine without causing allergic reactions.

Ethical approval

All procedure for Animal experiment has been approved by Ethics Commission of Brawijaya University.

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Author contribution

W and IM wrote the manuscript and participated in the study design. MR and FZH drafted and revised the manuscript. BP performed animal vaccination and flow-cytometry. W performed bioinformatics analyses and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflict of interests.

Research registration unique identifying number (UIN)

None.

Guarantor

None.

Consent to publish

This manuscript does not contains any individual person's data.

Consent to participate

This manuscript does not involving human participants, human data, or human tissue.

Availability of data and materials

We do not share the data in public repository, since we are using the data for patent application.

Provenance and peer review

Not commissioned, peer reviewed.

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