

Blockade of complement activation in bullous pemphigoid by using recombinant CD55-CD46 fusion protein

Pei Qiao, Yi-Xin Luo, Da-Long Zhi, Gang Wang, Er-Le Dang

Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China.

To the Editor: Bullous pemphigoid (BP) is an autoimmune blistering disease that is caused by autoantibodies and is associated with complement activation.^[1] Autoantibody-mediated complement activation is involved in BP pathogenesis. Complement-regulatory proteins (CRPs) including CD35, CD46, CD55, and CD59 comprise an important class of regulatory proteins in the complement system that control the enzyme cascades, assembly of the membrane-attack complex, and complement system homeostasis.^[2] Classically, both CD55 and CD46 negatively regulate complement system activation by inhibiting the production of new C3 and C5 convertases and accelerating the degradation of those already formed, thereby protecting cells from complement activation-induced damage. To date, the molecular fusion of different CRPs has been used to create chimeric molecules with novel functions.^[3] In this study, we generated a novel decay-accelerating factor and membrane cofactor protein (DAF-MCP, CD55-CD46) fusion protein to achieve a higher inhibitory activity against BP.

The amino acid and nucleotide sequences of CD55 and CD46 were analyzed and codon-optimized using ClustalW (version 2.1, UCD, Ireland), Vector NTI Viewer (version 4.0.1, BioScience Technology readers, USA) and National Center for Biotechnology Information (GenBank number: NM_000574.5, NM_002389.4). Both proteins have similar overall structures comprising one signal peptide, four complement control protein-repeat domains, and one serine/threonine-rich domain. To achieve greater inhibitory effects for BP, the coding sequences of both proteins were joined in-frame to produce a chimeric gene encoding the recombinant DM fusion protein [Figure 1A]. The fusion gene was ligated into the pET-28a expression vector through the *Nde* I and *Xho* I restriction sites to obtain the recombinant plasmid shown in Figure 1B; the primer P1 sequence for the primer site was 5'- ccaccatag-gactgtggccttccccagat-3' (*Nde* I restriction site underlined),

whereas the reverse primer P2 sequence was 5'- ccacctc-gagttatgagactggaggcttgaagtagg-3' (*Xho* I restriction site underlined). The linker sequence was 5'-ggcggaggaagtgggg-gaggatctggcggaggatct-3'. Polymerase chain reaction (PCR) was performed, and the PCR product was purified using a TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa BIO Inc., Dalian, China) according to the manufacturer's instructions, digested with *Nde* I and *Xho* I, and inserted into pET-28a that had been digested by the same enzymes. Accurate construction of the recombinant pET-28a-DM vector was confirmed using double-enzyme digestion and DNA sequencing (Life Technology Inc., Shanghai, China) as shown in Figure 1C. These data confirmed that the foreign gene was inserted correctly and recombinant plasmid (pET-28a-DM) was successfully constructed.

In addition, *E. coli* Top10 cells, transformed with the pET-28a-DM vector, were cultured in luria-bertani supplemented with 100 µg/mL kanamycin for 3 h. When the culture reached an $A_{600} = 0.6$, 0.5 mmol/L isopropyl-β-D thiogalactopyranoside (IPTG) (Sigma, St. Louis, MO, USA) was added to induce the expression of the DM fusion protein at 18 °C for 16 h. Because the fusion protein was mostly found in the precipitate, the inclusion bodies were dissolved in a dissolving buffer and purified using NI-NTA affinity chromatography (Qiagen, CA, USA) according to the manufacturer's instructions under native conditions by binding, washing, and elution. Finally, 1 mg of recombinant fusion protein with a purity of greater than 85% was obtained. As shown in Figure 1D-1E, the purified protein had a molecular weight of 71,000 as determined using 10% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, which is consistent with the expected value. Then, we validated this result via separate Western blots using monoclonal antibodies against CD55 and CD46 which was consistent with the SDS-PAGE data [Figure 1F].

Access this article online

Quick Response Code:



Website:

www.cmj.org

DOI:

10.1097/CM9.0000000000001312

Correspondence to: Prof. Er-Le Dang, Department of Dermatology, Xijing Hospital, Fourth Military Medical University, 127 Changle Western Road, Xi'an, Shaanxi 710032, China
E-Mail: dangerle@fmmu.edu.cn.

Copyright © 2020 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2021;134(7)

Received: 29-05-2020 Edited by: Ning-Ning Wang

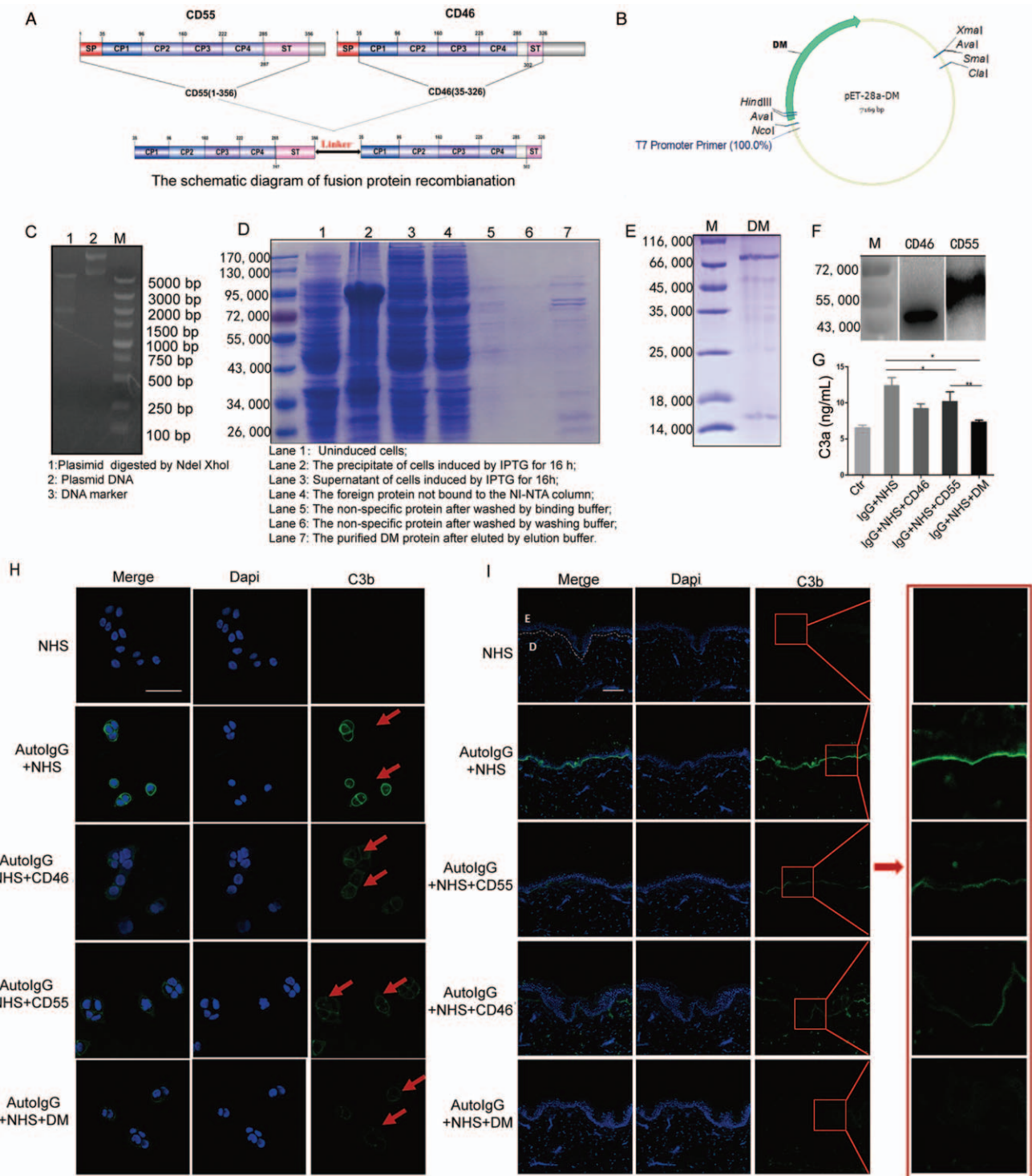


Figure 1: (A) Schematic diagram of fusion protein DM recombination. (B) The recombinant plasmid of DM obtained by mixing its genetic fragments with pET-28a through the *NdeI* and *XhoI* restriction sites. (C) Expected target band identified using colony PCR and 1% agarose gel electrophoresis. (D–F) Expression and purification of the DM protein using SDS-PAGE and Western blot. (G) C3a expression in the supernatant of HaCaT cells detected using ELISA. Fusion protein DM inhibits the autoantibody-mediated C3b deposition in HaCaT cells (H) and frozen skin sections (I) incubated with purified pathogenic auto-IgG from BP patients and fresh serum from healthy controls containing complement components, C3b deposition was detected using immunofluorescent staining to determine the complement activation level. Scale bar: 100 nm. Auto-IgG: pathogenic IgG from BP patients' serum; ELISA, Enzyme-linked immunosorbent assay; NHS: Normal human serum; SDS-PAGE, dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis. Results are representative of three independent experiments. Data are expressed as means and standard errors of the means.

To clarify the role of the DM protein in BP pathogenesis, we incubated HaCaT cells and healthy skin sections with pathogenic IgG from BP patients and fresh serum from healthy volunteers containing complement components, as

described previously.^[4] Immunofluorescence staining was performed 2 h later to evaluate C3b deposition. Clear C3b deposition was noted in HaCaT cells and Basement Membrane Zone (BMZ) of healthy skin sections incubated

with pathogenic IgG, and this effect was inhibited by treatment with 10 $\mu\text{g/mL}$ recombinant DM protein [Figure 1H-1I]. In addition, the group incubated with DM protein had higher inhibitory activity than the groups incubated with pathogenic IgG and either CD55 or CD46. Furthermore, based on enzyme-linked immunosorbent assay results, it was greatly inhibited following treatment of HaCatT cells with fusion protein compared with pathogenic IgG and either CD55 or CD46 [Figure 1G]. Consistent results were found in terms of C3b deposition. Statistical analysis was performed using GraphPad Prism software v5.0 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

Various studies have demonstrated that aberrant CD55/CD46 levels play roles in the pathophysiology of certain autoimmune diseases, such as SLE, rheumatoid arthritis, and myasthenia gravis. In our previous studies, it was a rare report that recombinant CD55 and CD46 could inhibit autoantibody-mediated complement activation, thus paving new directions for BP treatment.^[4,5] As the complement system is an attractive therapeutic target, we hope to generate a novel fusion protein composed of different CRPs to achieve higher inhibitory activity against BP. Thus, this study demonstrates that the constructed DM fusion protein could be a potential therapeutic direction against BP, further studies are needed to confirm this in a clinical setting.

Funding

This work was granted by the National Natural Science Foundation of China (No. 81703116, No. 81703125, and No. 81903208).

Conflicts of interest

None.

References

1. Zhao WL, Wang YM, Yuan J, Zeng YP, Li L. Alzheimer's disease identified in a patient with bullous pemphigoid by dementia screening scales. *Chin Med J* 2019;132:1619–1620. doi: 10.1097/CM9.0000000000000285.
2. Schmidt E, della Torre R, Borradori L. Clinical features and practical diagnosis of bullous pemphigoid. *Immunol Allergy Clin North Am* 2012;32:217–232. doi: 10.1016/j.iac.2012.04.002.
3. Lessey E, Li N, Diaz L, Liu Z. Complement and cutaneous autoimmune blistering diseases. *Immunol Res* 2008;41:223–232. doi: 10.1007/s12026-008-8028-y.
4. Qiao P, Dang E, Cao T, Fang H, Zhang J, Qiao H, *et al.* Dysregulation of mCD46 and sCD46 contribute to the pathogenesis of bullous pemphigoid. *Sci Rep* 2017;7:145. doi: 10.1038/s41598-017-00235-3.
5. Qiao P, Dang EL, Fang H, Zhang JY, Li B, Shen SX, *et al.* Decreased expression levels of complement regulator CD55 contribute to the development of bullous pemphigoid. *Oncotarget* 2018;9:35517–35527. doi: 10.18632/oncotarget.21216.

How to cite this article: Qiao P, Luo YX, Zhi DL, Wang G, Dang EL. Blockade of complement activation in bullous pemphigoid by using recombinant CD55-CD46 fusion protein. *Chin Med J* 2021;134:864–866. doi: 10.1097/CM9.0000000000001312