論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	Dianita Susilo Saputri
論文題名 Title	Deciphering the antigen specificities of antibodies by clustering their complementarity determining region sequences (相補性決定領域配列のクラスタリングによる抗体の抗原特異性の解読)

論文内容の要旨

〔目　的(Purpose)〕

Determining antigen and epitope specificity is an essential step in the discovery of therapeutic antibodies as well as in the analysis of adaptive immune responses to disease or vaccination. Despite extensive efforts, deciphering antigen specificity solely from antibodies or B cell receptors (BCR) amino acid sequence remains a challenging task, requiring a combination of experimental and computational approaches. Here, we describe and experimentally validate a simple and straightforward approach for grouping antibodies that share antigen and epitope specificities based on their complementarity determining region (CDR) amino acid sequence similarity.

〔方法ならびに成績(Methods/Results)〕

Using the proposed method (CDR clustering), we demonstrate that SARS-CoV-2 spike protein receptor binding domain (RBD)-binders and non-RBD binders from publicly available BCR data were classified correctly, with a cluster purity of 95%. These clusters were then leveraged for annotating unlabeled COVID-19 patient BCR data, enabling the discovery of novel anti-RBD antibodies as well as confirming the antigen specificity prediction by CDR clustering. We further validated the method by clustering BCR repertoires obtained from single-cell immune profiling of Diphtheria-Tetanus-Pertussis (DTP)-vaccinated donors. Antibody expression and antigen binding assays demonstrated that the clusters exhibited 96% antigen purity, surpassing the apparent 82% purity achieved by assigning antigens to the same B cells using fluorescently labeled DTP antigen probes. Moreover, antibodies within certain clusters were found to possess neutralizing activity, suggesting that CDR clusters contain epitope-level information.

〔総　括(Conclusion)〕

CDR clustering allows us to identify the specificities of a large number of antibodies whose antigen targets are unknown, using a small fraction of antibodies with well-annotated binding specificities. Together, this study offers a simple approach for antigen- and epitope-specific BCR discovery that is reproducible, inexpensive, and applicable to a wide range of antigen targets.

論文審査の結果の要旨及び担当者

（申請者氏名） Dianita Susilo Saputri

論文審査担当者		（職）	氏　名	
	主　査	大阪大学教授	Daron M. Standley	Daron Standley
	副　査	大阪大学教授	Tetsuya Iida	飯田哲也
	副　査	大阪大学教授	Wataru Ise	伊勢渉

論文審査の結果の要旨

In this study, Dianita (Nita) Saputri aimed to address the specificity of B cell receptors (BCRs) by using a machine learning tool capable of transluting diverse sequences into similar functional similarities. The study focused on Diphtheria-Tetanus-Pertussis (DTP) bacterial toxoids as model antigens. Vaccinating four donors with DTP, she isolated and sequenced B cells, grouping them based on reactivity against each antigen. A tool was developed to cluster BCR sequences targeting a single antigen at a specific binding site. Initially, despite her initial expectations, the results took an unexpected turn, revealing apparently mixed-specificity BCR clusters. However, through meticulous experimentation, Nita demonstrated that these seemingly impure clusters were, in fact, 95% pure in terms of targeted antigen. Notably, the clustering tool successfully identified groups of BCRs targeting the same epitope and antigen. Despite encountering unexpected challenges in the cell sorting step, such as false positives from "sticky" fluorescently labeled antigens, Nita's persistence and thorough validation efforts led to the acceptance of our paper in mSystems 2023. In conclusion, the study demonstrates the effectiveness of machine learning based on paratope features in translating diverse immune receptor sequences. Nita's remarkable ability to independently design and execute experiments showcased her scientific acumen, adaptability, and organizational skills. She also co-authored several review papers on BCR repertoire analysis. This success not only reflects Nita's capabilities but also highlights the potential for widespread recognition of her contributions. Nita's exceptional performance and the project's success underscore her potential to excel in various laboratory environments. I believe that her skills and achievements will allow her to succeed in both research and clinical studies. This research is worth being granted a doctoral degree (medicine).