"Alignmentaj" — a tool for *in silico* prediction of similar peptides potentially involved in molecular mimicry phenomenon (short communication)*

M. G. Normatov, V. A. Pakhomov, L. P. Churilov

St. Petersburg State University,
7–9, Universitetskaya nab., St. Petersburg, 199034, Russian Federation

For citation: Normatov M. G., Pakhomov V. A., Churilov L. P. "Alignmentaj" — a tool for *in silico* prediction of similar peptides potentially involved in molecular mimicry phenomenon (short communication). *Vestnik of Saint Petersburg University. Medicine*, 2025, vol. 20, iss. 1, pp. 96–101. EDN OQEMMA

Global burden of autoimmune pathology is getting heavier with every year. The antigenic mimicry of host and alien peptides may provoke pathological autoimmunity in individuals with some alleles of HLA. Identification of peptides shared between host and pathogen proteins can be a useful technique for studying molecular mimicry, evaluation of autoimmunity risks, and for selecting epitopes while developing the safest vaccines. There are many examples of the autoimmune response induction due to antigenic mimicry, the recent ones concern the new Coronavirus infection COVID-19 as a trigger of autoimmunity. The pentapeptides are proven to be minimal sequential immunogenic protein epitopes. Therefore, we created a new computer program "Alignmentaj" that identifies similar pentapeptides between two proteins, for example autoantigens and antigens of infectious agents. The resulting model has been implemented in a revised version of the "Alignmentaj" server (https://muslimb.github.io/ProjectAlignmentaj/index.html) and successfully used to explore molecular mimicry of human and viral antigens. The principle of its work is described and perspectives of bioinformatic analysis in immunology discussed.

Keywords: autoimmunity, bioinformatics, epitope, molecular mimicry, pentapeptide, vaccine.

Introduction: Autoimmune Diseases and Molecular Mimicry

Autoimmune diseases are a group of illnesses in which autoreactive immune cells and/or autoantibodies attack the self targets. They can be regarded as an exaggeration of physiological autoimmune mechanisms or as a kind of "autoallergy", which means a hyperactive, wrong targeted and poorly regulated autoimmunity [1]. At least 3–5 % of people suffer from autoimmune diseases in some populations [2; 3]. The global burden of autoimmune pathology is on the rise, making this group of diseases third biggest reason of death and disability in many populations, especially among women [4]. The countries with higher sociodemographic development levels shouldered disproportionately higher burden of pathological autoimmunity, which inequality gets more noticeable with every year [5]. Autoimmune disorders have multi-factorial etiology, however the main factors are genetic predisposition [6] and hyperstimulation of the immune system by the environ-

* The work was supported by the grant of the Russian Science Foundation, no. 22-15-00113, https://rscf.ru/project/22-15-00113.

mental and/or endogenous agents [7–10]. Infectious agents have long been the most well-studied environmental factors altering autoimmunity by various mechanisms [11; 12]. Molecular mimicry underlies the pathogenesis of autoimmune diseases associated with microorganisms [12; 13]. The term "molecular mimicry" was first coined by an American biologist Raymond T. Damain in 1964 [14; 15]. But the very existence of common proteins in microorganisms and higher eukaryotes was predicted much earlier by Russian evolutionary biologist Konstantin Sergeevich Merezhkovsky (1855–1921), an alumnus of Saint Petersburg University, while developing his symbiogenetic theory of the cell origin [16]. Molecular mimicry occurs when there are epitopes shared between antigens of a pathogen and human autoantigens. Potentially it may be the cause of autoimmune disorders, if the host immune system, being restricted by certain HLA haplotype, will process the alien antigen using shared epitopes for antigen presentation [17]. In this case anti-alien T-helpers may boost anti-self response. The way of autoimmune disorder provocation *via* the antigenic mimicry is illustrated in Figure 1. Mechanisms of autoimmunity provocation in molecular mimicry: 1) antigens of viruses are recognized by antigen-presenting cells (APCs); 2) APCs presenting mimicking peptide to T-helper cells; 3a) B cells or 3b) cytotoxic T cells activated by T-helper; 4a) B cells produce autoantibodies against self proteins or 4b) own cells are destroyed by cytotoxic T-lymphocytes.

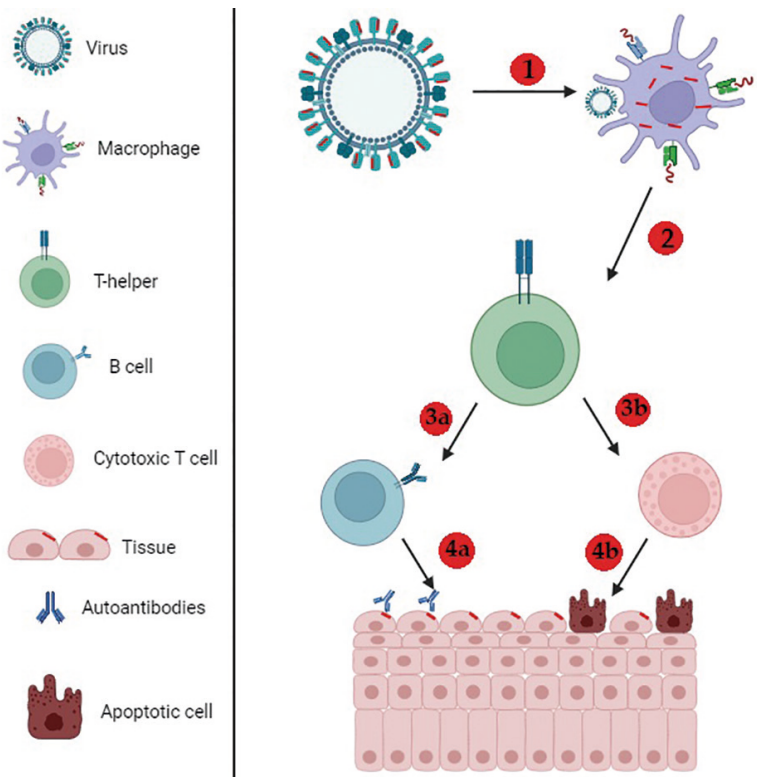

Fig. 1. The way of autoimmune disorder provocation *via* the antigenic mimicry.
The picture was created using the BioRender web-server (https://www.biorender.com/)

Epitope spreading in autoimmunity was first noted while modeling experimental autoimmune encephalomyelitis [18]. Later it was demonstrated in many experimental and clinical diseases [12; 15].

The Immune Epitope Database (IEDB) is a freely available resource of experimental data on antibody and T cell epitopes studied in humans and other animal species in the context of infectious disease, allergy, autoimmunity and transplantation [19]. The IEDB recently was used by us and other authors for prediction of mimicking potential of SARS-CoV-2 epitopes regarding the most essential target autoantigens of human endocrinopathies, with appropriate pathomorphological and clinical correlates documented COVID19-mediated triggering of autoimmune endocrine diseases [20–22].

Implementation

The minimal recognizable sequential antigenic determinant is a pentapeptide [23]. Hence, we created, registered in Russian Federation Intellectual Property Service (Registration certificate N 2023617186) and implemented an original computer program named "Alignmentaj" searching the shared pentapeptides in protein antigens. "Alignmentaj" is written in the Python programming language. Our program source code is available in https://github.com/muslimb/MyProekt1 and web tool in https://muslimb.github.io/ProjectAlignmentaj/index.html. "Alignmentaj" currently supports reading popular biological data format FASTA with amino acid sequences of protein. Algorithms of our program operation are illustrated in Figure 2 (the picture was created using the Microsoft Word 2016 v. 2109). "Alignmentaj" web tool algorithm. Input information: 1) the length of the peptide to be analyzed can be entered at the tool input; 2) amino acid sequences of

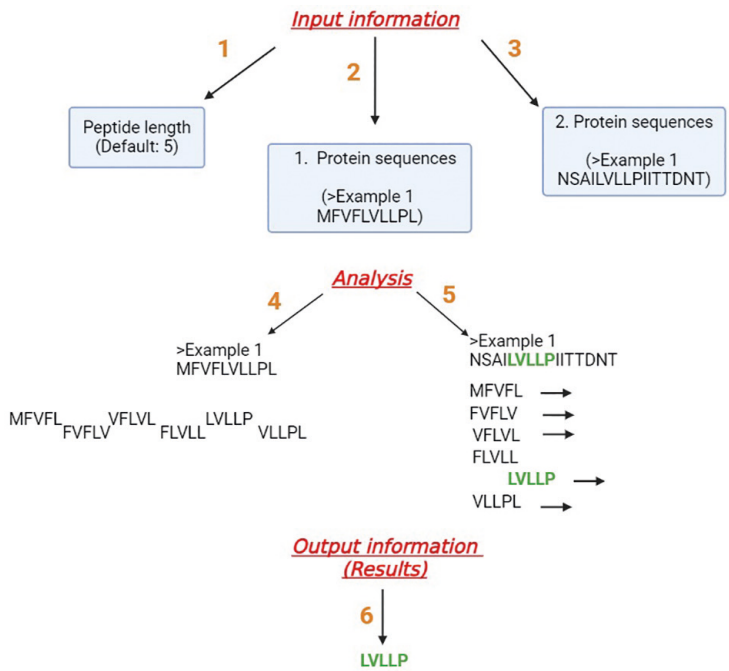

Fig. 2. "Alignmentaj" web tool algorithm

the 1st protein; 3) amino acid sequences of the 2nd protein. Analysis: 4) the 1st protein primary sequence dissected into pentapeptides offset by one residue (i. e., MFVFL, FVFLV, VFLVL, FLVLL, etc.), and the resulting 1st pentapeptides are: 5) analyzed for occurrences within the 2nd protein by the web tool "Alignmentaj". Output information (Results): 6) if there are similar peptides in the analyzed proteins, we get them in the output.

Conclusions

Bioinformatics approach is considered essential in analysis of autoimmune diseases [24]. The prediction of similar peptides between infectious antigens and human autoantigens has been extensively studied by a number of authors in last years [25–27]. That's why, we have created a web tool and program "Alignmentaj" for searching shared peptides between human and microbial/viral proteins. It is based on the alignment of amino acid sequences between two proteins. We consider bioinformatic analysis prediction of molecular mimicry to be an essential step in the preliminary evaluation of autoimmunity risks. The program can be used in the field of medicine (infectiology, immunology and allergology) and also is applicable in selecting epitopes for the development of the safest vaccines.

References

1. Poletaev A. B., Churilov L. P., Stroev Y. I., Agapov M. M. Immunophysiology versus immunopathology: Natural autoimmunity in human health and disease. *Pathophysiology*, 2012, vol. 19, pp. 221–231.
2. Jacobson D. L., Gange S. J., Rose N. R., Graham N. M. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol*, 1997, vol. 84, pp. 223–423.
3. Eaton W. W., Rose N. R., Kalaydjian A., Pedersen M. G., Mortensen P. B. Epidemiology of autoimmune diseases in Denmark. *J Autoimmun*, 2007, vol. 29, pp. 1–9.
4. Miller F. W. The increasing prevalence of autoimmunity and autoimmune diseases: An urgent call to action for improved understanding, diagnosis, treatment, and prevention. *Current Opinion in Immunology*, 2023, vol. 80, p. 102266.
5. Cao F., He Y. S., Wang Y., Zha C. K., Lu J. M., Tao L. M., Jiang Z. X., Pan H. F. Global burden and cross-country inequalities in autoimmune diseases from 1990 to 2019. *Autoimmun Rev*, 2023, vol. 22, p. 103326.
6. Ulmanen I., Halonen M., Ilmarinen T., Peltonen L. Monogenic autoimmune diseases — lessons of self-tolerance. *Curr Opin Immunol*, 2005, vol. 17, pp. 609–615.
7. Colafrancesco S., Perricone C., Priori R., Valesini G., Shoenfeld Y. Sjogren's syndrome: Another facet of the autoimmune/inflammatory syndrome induced by adjuvants (ASIA). *J Autoimmun*, 2014, vol. 51, pp. 10–16.
8. Kivity S., Arango M. T., Ehrenfeld M., Tehori O., Shoenfeld Y., Anaya J. M., Agmon-Levin N. Infection and autoimmunity in Sjogren's syndrome: A clinical study and comprehensive review. *J Autoimmun*, 2014, vol. 51, pp. 17–22.
9. Peng J., Narasimhan S., Marchesi J. R., Benson A., Wong F. S., Wen L. Long term effect of gut microbiota transfer on diabetes development. *J Autoimmun*, 2014, vol. 53, pp. 85–94.
10. Ryabkova V. A., Churilov L. P., Shoenfeld Y. Hyperstimulation of the immune system as a cause of autoimmune diseases. *Vestnik Rossiiskoi akademii meditsinskikh nauk*, 2020, vol. 75, pp. 204–213. (In Russian)
11. Bogdanos D. P., Smyk D. S., Invernizzi P., Rigopoulou E. I., Blank M., Pouria S., Shoenfeld Y. Infectome: A platform to trace infectious triggers of autoimmunity. *Autoimmun Rev*, 2013, vol. 12, pp. 726–740.
12. *Infection and Autoimmunity*. 3rd ed. N. Mahroum, A. Watad, Y. Acad Shoenfeld (eds). London: Elsevier, 2024. 1120 p.
13. Rojas M., Restrepo-Jiménez P., Monsalve D. M., Pacheco Y., Acosta-Ampudia Y., Ramírez-Santana C., Leung P. S. C., Ansari A. A., Gershwin M. E., Anaya J. M. Molecular mimicry and autoimmunity. *J Autoimmun*, 2018, vol. 95, pp. 100–123.

14. Damian R. T. Molecular Mimicry: Antigen Sharing by Parasite and Host and Its Consequences. *American Naturalist*, 1964, vol. 98, pp. 129–149.
15. Blank M., Barzilai O., Shoenfeld Y. Molecular mimicry and auto-immunity. *Clin Rev Allergy Immunol*, 2007, vol. 32, pp. 111–118.
16. Mereschkowsky K. Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. *Biol Centralbl*, 1910, vol. 30, pp. 353–367.
17. Getts D. R., Chastain E. M., Terry R. L., Miller S. D. Virus Infection, Antiviral Immunity, and Autoimmunity. *Immunol Rev*, 2013, vol. 255, pp. 197–209.
18. Lehmann P. V., Forsthuber T., Miller A., Sercarz E. E. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature*, 1992, vol. 358, pp. 155–157.
19. Vita R., Overton J. A., Greenbaum J. A., Ponomarenko J., Clark J. D., Cantrell J. R., Wheeler D. K., Gabbard J. L., Hix D., Sette A., Peters B. The immune epitope database (IEDB) 3.0. *Nucleic Acids Res*, 2015, vol. 43 (D1), pp. D405–412.
20. Normatov M. G., Karev V. E., Kolobov A. V., Mayevskaya V. A., Ryabkova V. A., Utekhin V. J., Churilov L. P. Post-COVID Endocrine Disorders: Putative Role of Molecular Mimicry and Some Pathomorphological Correlates. *Diagnostics*, 2023, vol. 13, p. 522.
21. Churilov L. P., Normatov M. G., Utekhin V. J. Molecular Mimicry between SARS-CoV-2 and Human Endocrinocytes: A Prerequisite of Post-COVID-19 Endocrine Autoimmunity? *Pathophysiology*, 2022, vol. 29, pp. 486–494.
22. Wang Y., Guo H., Wang G., Zhai J., Du B. COVID-19 as a Trigger for Type 1 Diabetes. *The Journal of Clinical Endocrinology and Metabolism*, 2023, vol. 108, pp. 2176–2183.
23. Kanduc D. On the Pentapeptide as the Measurement Unit in Immunology. *Glob Med Genet*, 2024, vol. 11, pp. 034–035.
24. Danieli M. G., Brunetto S., Gammeri L., Palmeri D., Claudi I., Shoenfeld Y., Gangemi S. Machine learning application in autoimmune diseases: State of art and future prospectives. *Autoimmun Rev*, 2023, vol. 23, p. 103496.
25. Lerner A., Benzvi C., Vojdani A. SARS-CoV-2 Gut-Targeted Epitopes: Sequence Similarity and Cross-Reactivity Join Together for Molecular Mimicry. *Biomedicines*, 2023, vol. 11, p. 1937.
26. Adiguzel Y., Shoenfeld Y. Shared 6mer Peptides of Human and Omicron (21K and 21L) at SARS-CoV-2 Mutation Sites. *Antibodies*, 2022, vol. 11, p. 68.
27. Churilov L. P., Kanduc D., Ryabkova V. A. COVID-19: Adrenal response and molecular mimicry. *The Israel Medical Association Journal*, 2021, vol. 23, pp. 618–619.

Received: March 11, 2024
Accepted: December 27, 2024

Authors' information:

Muslimbek G. Normatov — Postgraduate Student; https://orcid.org/0000-0002-1307-0513, muslimbek_normatov@mail.ru
Vladimir A. Pakhomov — Research Engineer; https://orcid.org/0009-0001-8452-1238, pva-123@yandex.ru
Leonid P. Churilov — PhD in Medicine, Associate Professor; https://orcid.org/0000-0002-9807-6606, l.churilov@spbu.ru

«Alignmentaj» — инструмент для предсказания *in silico* сходных пептидов, потенциально участвующих в феномене молекулярной мимикрии (краткое сообщение)*

М. Г. Норматов, В. А. Пахомов, Л. П. Чурилов

Санкт-Петербургский государственный университет,
Российская Федерация, 199034, Санкт-Петербург, Университетская наб., 7–9

Для цитирования: *Normatov M. G., Pakhomov V. A., Churilov L. P.* "Alignmentaj" — a tool for *in silico* prediction of similar peptides potentially involved in molecular mimicry phenomenon (short communication) // Вестник Санкт-Петербургского университета. Медицина. 2025. Т. 20. Вып. 1. С. 96–101. EDN OQEMMA

Глобальное бремя аутоиммунной патологии с каждым годом становится все тяжелее. Антигенная мимикрия пептидов хозяина и чужеродных пептидов может провоцировать патологический аутоиммунитет у лиц с некоторыми аллелями HLA. Идентификация пептидов в белках, общих для белков хозяина и патогена, может быть полезным методом для изучения молекулярной (антигенной) мимикрии, оценки риска аутоиммунных нарушений и выбора эпитопов при разработке более безопасных вакцин. Существует множество примеров индукции аутоиммунного ответа за счет антигенной мимикрии, последние из них касаются новой коронавирусной инфекции COVID-19 как триггера аутоиммунитета. Доказано, что именно пентапептиды представляют собой минимальные секвенциальные иммуногенные белковые эпитопы для иммунных клеток человека. Поэтому мы создали, зарегистрировали и применили оригинальную компьютерную программу «Alignmentaj», которая идентифицирует сходные пентапептиды между двумя белками, например аутоантигенами человека и антигенами инфекционных агентов. Полученная модель была реализована в обновленной версии сервера "Alignmentaj" (https://muslimb.github.io/ProjectAlignmentaj/index.html) и успешно использована для изучения молекулярной мимикрии человеческих и вирусных антигенов. В сообщении описан принцип работы данной программы и обсуждены перспективы применения биоинформационного анализа в иммунологии.

Ключевые слова: аутоиммунитет, биоинформатика, эпитоп, молекулярная мимикрия, пентапептид, вакцина.

Статья поступила в редакцию 11 марта 2024 г.;
рекомендована к печати 27 декабря 2024 г.

Контактная информация:

Норматов Муслимбек Гуломович — аспирант; https://orcid.org/0000-0002-1307-0513, muslimbek_normatov@mail.ru
Пахомов Владимир Анатольевич — инженер-исследователь; https://orcid.org/0009-0001-8452-1238, pva-123@yandex.ru
Чурилов Леонид Павлович — канд. мед. наук, доц.; https://orcid.org/0000-0002-9807-6606, l.churilov@spbu.ru

* Работа поддержана грантом Российского научного фонда № 22-15-00113, https://rscf.ru/project/22-15-00113.