Immunity
Meeting Report

A Case for a Human Immuno-Peptidome Project Consortium

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A multidisciplinary group of researchers gathered at the Hönggerberg Campus at ETH Zurich, Switzerland, for the first meeting on the Human Immuno-Peptidome Project (https://hupo.org/human-immuno-peptidome-project/). The long-term goal of this project is to map the entire repertoire of peptides presented by human leukocyte antigen molecules using mass spectrometry technologies, and make its robust analysis accessible to any immunologist. Here we outline the specific challenges identified toward this goal, and within this framework, describe the structure of a multipronged program aimed at addressing these challenges and implementing solutions at a community-wide level. Pillars of that program are: (1) method and technology development, (2) standardization, (3) effective data sharing, and (4) education. If successful, this community-driven endeavor might provide a roadmap toward new paradigms in immunology.

Challenges

Following the completion of the Human Genome Project, advances in mass spectrometry (MS) technologies over the last decade, and the recent breakthroughs in immuno-oncology, MS profiling of HLA-associated peptides—referred herein as immunopeptidomics—has become a dynamic new frontier in immunology, vaccines, and immunotherapy. Notably, the capability of current MS technologies to identify thousands of HLA-associated peptides from human tissues, including tumor-specific mutant neo-antigens, has attracted a high level of interest, because their robust identification will have a positive impact in precision medicine (Bassani-Sternberg et al., 2016). Moreover, the importance of investigating the immunopeptidome using MS technologies has recently been endorsed by 35 of the world's leading vaccines researchers in the context of the Human Vaccines Project (HVP) (http://www.humanvaccinesproject.org/) (Koff et al., 2014; Sette et al., 2016). Thus, the basic science and clinical need for the comprehensive analysis of the immunopeptidome has been well recognized. Nevertheless, persistent technical limitations have historically prevented the full deployment and usage of immunopeptidomics tools in immunology. In fact, the analysis of immunopeptidomes using MS technologies is a relatively complex process requiring three main components: first, isolation of HLA-associated peptides from cells or tissues by immunoaffinity purification; second, acquisition of the isolated peptides using a discovery or targeted MS method; and finally, computational analysis of the acquired peptides using specialized software tools. Currently, the main challenges in the field are related to these three steps and can be summarized as follow (also highlighted in Box 1): (1) uncertainty about the yield of the peptide isolation procedure, (2) inability to analyze immunopeptidomes from small amount of biological materials (e.g., tissue needle biopsy), (3) sample throughput, (4) cost, sensitivity, and reproducibility of MS technologies, (5) suboptimal identification rate of high confidence HLA-associated peptides, (6) lack of high quality experimental and computational standards, and (7) poor accessibility to large volume of MS data generated by the community. In addition, it is recognized that interested non-expert groups cannot quickly jump into the field as they generally struggle for months with the technicalities of setting up a proper experimental and analytical workflow and with generating high quality and reproducible research results. Consequently, the field is expanding slowly and very few laboratories worldwide have developed the required technical expertise to generate very high quality immunopeptidomic datasets.

To further accelerate research toward comprehensive and reproducible analysis of immunopeptidomes, leaders in immunopeptidomics in 2015 created a Human Immuno-Peptidome Project (HIPP) as a new initiative under the umbrella of the Human Proteome Organization (HUPO) (https://hupo.org/human-immuno-peptidome-project/). The HUPO-HIPP initiative was launched with the vision of making data and robust experimental and computational techniques of immunopeptidomics accessible to any immunologist, clinical investigator, and other researchers, to increase the impact of immunopeptidomics in biomedical research on a global scale. Given the complexity of the challenges described above, those involved in founding the HUPO-HIPP initiative proposed to form a working group to address and discuss these issues in greater depth.

On May 4–5th, 2017, 40 leading scientists and industry representatives from 18 universities and 9 companies convened in Zurich, Switzerland, for the first international HUPO-HIPP workshop (Figure 1). The primary goal of this workshop was to bring together leading experts in MS, proteomics, computational sciences and immunology to discuss the state of the art in

Box 1. Challenges in Immunopeptidomics and Recommended Actions

Challenge	Pillar of the HUPO-HIPP program	Specific recommendation and implementation (if applicable)
Uncertainty about the yield of the peptide isolation procedure	M&T development	We plan to create a large library of isotopically labeled peptide-MHC monomers for the accurate quantification of stepwise yields of the procedure (Hassan et al. 2014). The library will be distributed to different groups to assess the interlaboratory reproducibility of the procedure.
Inability to analyze immunopeptidomes from small amount of biological materials (e.g. tissue needle biopsy)	M&T development	N.A.
Low sample throughput	M&T development	N.A.
Limited sensitivity of MS instruments	M&T development	N.A.
Suboptimal identification rate of high-confidence HLA-associated peptides	M&T development	HUPO-HIPP plans to partner with MS developers (e.g. Thermo Scientific) to further improve the EThcD fragmentation method. We also plan to initiate a benchmarking study using a large collection of synthetic HLA-associated peptides to assess the performance of computational tools.
Limited reproducibility of MS measurements	M&T development	We will promote and teach SWATH-MS/DIA methods for reproducible analysis of immunopeptidomes. HLA allele-specific spectral libraries will be continuously generated and deposited into the SysteMHC Atlas to further develop and deploy SWATH-MS/DIA methods across the community and to enable the reproducible measurement of sample cohorts in the future.
Lack of consistent and informative documentation for standardized reporting of immunopeptidomics studies	Standardization	We have created specific working groups to design guidelines that specify what information must be included in a submitted manuscript. HUPO-HIPP will also report the Minimal Information about Immuno-Peptidomics Experiment (MIAIPE) to standardize data annotation and foster effective data sharing.
Poor accessibility to published immunopeptidomic datasets generated by MS	Effective data sharing	HUPO-HIPP plans to seek partnerships with scientific journals and funding agencies to enforce data sharing. We will also further develop the SysteMHC Atlas and improve its interoperability with PRIDE, SWATH Atlas and IEDB.
Small community and technical challenges in establishing a proper immunopeptidomic workflow	Education	HUPO-HIPP has formed a committee for organizing the first immunopeptidomics summer school. The course will cover both experimental and computational procedures and will take place in 2018.

Not applicable (N.A.), Method and technology (M&T).

immunopeptidomics. Specifically, the workshop aimed at discussing the challenges described above, outlining specific recommendations and how they could be best tested and implemented (Box 1).

Structure of the Hupo-Hipp Program and Recommended Next Steps

To solve the challenges described above, the working group concluded that the HUPO-HIPP program would be structured into four main pillars: (1) method and technology development, (2) standardization, (3) effective data sharing, and (4) education (Box 1). Participants also endorsed the creation of a large-scale immunopeptidome consortium that would provide a suitable platform to leverage the HUPO-HIPP program and translate it into deliverable actions. Below we summarize the topics discussed and recommendations arising from the workshop.

Method and Technology Development

Clinical investigators at the workshop stressed the need to improve the performance of MS-based immunopeptidomic workflows as the size of particularly informative human samples (e.g., needle biopsies/1 mg) are generally significantly smaller than the samples required for analysis by current techniques. In contrast, relatively large amounts of tissues (~1 g or 1 cm^3) are currently needed to properly characterize the immunopeptidome of human samples. The development of rapid and efficient techniques for processing and analyzing small tissue samples is

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Figure 1. Participants in the Human Immuno-Peptidome Project workshop held in Zürich, Switzerland, on May 4 and 5, 2017

therefore crucial for the robust analysis of immunopeptidomes and will be necessary to scale up the process to advance the field effectively into routine clinical application.

Method developers argued at the workshop that the peptide isolation procedure is likely to be the Achilles heel in the whole immunopeptidomics workflow. For instance, in other -omic fields, sample preparation protocols ideally enable all sample processing steps to be carried out in a single tube to minimize sample losses, thereby enhancing sensitivity, throughput and scalability of analyses. However, in immunopeptidomics, the immunoaffinity purification method—which enables the isolation of HLA-associated peptides with a high level of specificity—generally requires multiple steps, relatively large amounts of antibodies, and was shown to come with the cost of a very low yield, i.e., 1%–3% (Hassan et al., 2014), although this observation is currently debated and would still need to be reproduced in several settings. To properly assess the intra- and inter-laboratory reproducibility of available immunoaffinity purification methods, HUPO-HIPP proposes to design a multi-laboratory study in which a large library of isotopically labeled peptide-MHC monomers will be distributed across different research groups for the accurate quantification of stepwise yields of the procedure using a targeted MS method (Box 1). Results from this joint project will clarify the uncertainty about the yield of the peptide isolation procedure, determine the robustness of the method in different groups and possibly indicate the need to prioritize the development of innovative sample preparation protocols.

Leaders in MS technologies also mentioned that a state of the art method for efficient fragmentation of HLA-associated peptides, so-called EThcD, enhances the identification success rate of HLA-associated peptides compared to other fragmentation methods by about 3-fold (Mommen et al., 2014). However, this fragmentation method is not fully suitable for the identification of peptides isolated from small amount of tissues. To overcome this limitation, we propose to establish a partnership between HUPO-HIPP and Thermo Scientific to further improve this method toward rapid and highly precise identification of HLA-associated peptides from tiny amount for biological materials.

It was also highlighted during the workshop that the most recent MS acquisition method—known as data-independent acquisition—is highly reproducible for the measurement of HLA-associated peptides across several samples (Caron et al., 2015), and, if further developed and deployed by the community, could represent a powerful strategy to support the consistent measurement of immunopeptidomic sample cohorts and foster democratization of the field. To this end, we will promote and teach this method by integrating specific teaching materials within our emerging educational program—i.e., the fourth pillar of the HUPO-HIPP initiative.

Altogether, we envision that comprehensive and quantitative mapping of immunopeptidomes should become as sensitive and reproducible as it is now for other revolutionizing high-throughput technologies in life sciences such as next-generation DNA and RNA sequencing. The combination of robust and scalable peptide isolation procedures, more sensitive and reproducible MS techniques, as well as benchmarked software tools for precise identification of HLA-associated peptides should increase the accessibility and impact of immunopeptidomics for the benefit of all. HUPO-HIPP might provide the vision to create such opportunities, and—akin to the Human Genome Project—represents a fresh platform to catalyze the development of new technologies.

Standardization

Standardization of data reporting and annotation is of great importance for any data-driven research community as it enables unambiguous interpretation and reproducibility of the data between several laboratories. In order to improve interpretation, accessibility, and reproducibility of immunopeptidomics

Box 2. Function of Specific Databases that Host MHC Peptide Datasets

Database	Main community targeted	Function
PRIDE	Proteomics	Established public repository for proteomics data generated by MS. The database includes raw and/or converted MS output files, protein and peptide identification, post-translational modification and spectral evidence. PRIDE is a core member in the ProteomeXchange consortium (http://www.proteomexchange.org/), which provides a single point for submitting MS-based proteomics data to public-domain repositories. Datasets are submitted to PRIDE via ProteomeXchange and are handled by expert biocurators (https://www.ebi.ac.uk/pride/archive/).
IEDB	Immunology	Established repository of experimental data characterizing antibody and T cell epitopes studied in humans, non-human primates, and other animal species. Epitopes involved in infectious disease, allergy, autoimmunity, and transplant are included. Besides data generated by various cell-based assays, IEDB hosts datasets of naturally presented MHC peptides obtained by MS analysis. List of human-readable MHC peptide sequences are extracted from the literature and are handled by expert curators. Raw MS output files are not available (http://www.iedb.org/).
SWATH Atlas	Proteomics Immunopeptidomics	Public resource in development that contains high-quality spectral libraries for the analysis of proteomics data generated by SWATH-MS/DIA. The Atlas includes libraries for analysis of proteomes of several species as well as HLA allele-specific spectral libraries for analysis of human immunopeptidomes. SWATH Atlas supports the robust measurement of sample cohorts. Raw MS output files are not available (http://www.swathatlas.org/)
SysteMHC Atlas	Immunopeptidomics Immunology	Public repository in development that aims at collecting, organizing, and sharing immunopeptidomic data generated by MS. The Atlas includes raw MS output files, context-specific datasets of MHC class I and class II peptides, standardized MHC allele-specific peptide spectral libraries, and links to PRIDE, IEDB, and SWATHAtlas. The SysteMHC Atlas serves as a community resource towards the generation of a high-quality comprehensive map of the human immunopeptidome and the support of consistent SWATH-MS/DIA-based measurement of immunopeptidomic sample cohorts (https://systemhcatlas.org/).

data, the immunology community will need specific annotation standards. With this in mind, the workshop participants plan to draft guidelines and report the Minimal Information about Immuno-Peptidomics Experiment (MIAIPE), which would describe specific information about sample type and preparation (e.g., organism, tissue and cell type, amount of sample, culture conditions, disease state, HLA type, antibody used for peptide isolation, etc.), LC-MS/MS acquisition parameters, informatics, and statistics. As a next step, HUPO-HIPP plans to establish a partnership with scientific journals and approach funding organizations to promote this community effort and to encourage deposition of properly annotated immunopeptidomic data in public repositories. In longer-term, this effort is expected to greatly increase the impact of shared data in public repositories and the potential large-scale integration and reanalysis of thousands of immunopeptidomics datasets to enable the generation of new and robust systems-level hypothesis in immunology.

Effective Data Sharing

Public repositories are essential infrastructures for successful sharing of genomic, transcriptomic, and proteomic datasets and support the re-uses of data that were originally acquired at high cost. HUPO-HIPP recognized the need for an open immunopeptidomics repository in which MS output files would be properly annotated, stored, and shared without restriction, and catalyzed the SysteMHC Atlas project (https://systemhcatlas.org), which was introduced at the workshop. The SysteMHC Atlas includes raw mass spectrometer output files collected from multiple laboratories, a catalog of context-specific datasets of HLA class I and class II peptides, standardized HLA allele-specific peptide spectral libraries consisting of consensus spectra, and link to other complementary proteomics and immunology databases such as PRoteomics IDEntifications (PRIDE) (https://www.ebi.ac.uk/pride/), SWATHAtlas (http://www.swathatlas.org/), and the Immune Epitope Database (IEDB) (http://www.iedb.org/) (Box 2). Given the number of additional immunopeptidomics-related databases that might be developed in the future, participants also discussed the importance of developing approaches to ensure interoperability of data repositories for the benefit of the end user. As a first step in this direction, we propose that the SysteMHC Atlas should consider becoming a member of the Proteome Exchange Consortium (http://www.proteomexchange.org/)—a truly collaborative and integrated platform that shares contents of multiple databases of which each database has its own specific focus.

Rapid deposition and effective sharing of large datasets has become the norm in the field of genomics and proteomics. However, widespread adoption of this practice has not been fully achieved in immunopeptidomics. As a result, valuable experimental immunopeptidomics MS data cannot serve a larger community of researchers to enable better collaborations and advance the field more efficiently. When questioned on this topic, participants at the workshop recognized the importance of expanding the incentives for effective deposition and sharing of immunopeptidomic MS data to the public domain. The working group emphasized that journals and funding agencies represent the most effective incentive to make data public. For

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instance, in 2014, the National Institute of Health (NIH) started implementing an expansive Genomic Data Sharing Policy, which requires that almost all large-scale genomics data generated or analyzed using NIH funds be shared. Given the potential huge benefit of data sharing in immunopeptidomics, HUPO-HIPP will explore opportunities with NIH, European Commission (EC) as well as with editors of scientific journals, and discuss approaches to enforce deposition of immunopeptidomic MS data in public repositories to promote a culture of open sharing in the field. Members of HUPO-HIPP will discuss this topic more thoroughly this September at the annual HUPO conference in Dublin, Ireland.

Education and Training Platforms

Participants acknowledged that the field has expanded very slowly over the last decades and also recognized that there was a lack of teaching material to bring interested groups quickly into the field. Therefore, the working group concluded that the time was right to create an international training program with the goal of building a larger community to more rapidly tackle the challenges described above. This approach has been very successful in the field of proteomics, as exemplified by the MaxQuant summer school (http://summerschool.maxquant.de/), the targeted proteomics course (http://dia-swath-course.ethz.ch/videos.html), and the TransProteomic Pipeline training course (http://www.proteomecenter.org/course.php), all of which providing hands-on training on analysis of proteomics data. Ideally, an international training program in immunopeptidomics would enable any interested group to rapidly access new technologies and knowledge for the isolation, identification and quantification of HLA-associated peptides, thereby accelerating dissemination of knowledge for efficient implementation of cutting-edge tools and methods into new research laboratories. This training program should include summer schools, theoretical courses, workshops, movie protocols, and internet forums. Notably, several members of HUPO-HIPP have already started to design the first immunopeptidomics summer school. Though the site has yet to be determined, the first summer school will cover both experimental and computational procedures and will most likely take place toward the end of 2018. HUPO will call for participants via social media post and direct people toward the HIPP webpage for registration. For the first year, the organizing committee aims at proactively selecting a limited number of attendees. Progress toward the stated goals of the initiative will be assessed by requesting feedback from the participants following the summer course. In longer term, HUPO-HIPP proposes to establish and coordinate training platforms at several sites worldwide to quickly bring talented people into the field to establish a large, strong, and dynamic international community.

Consortium

Inspired by success stories and key lessons from 25 years of big biology (Green et al., 2015), participants were asked whether a funded human immunopeptidome project consortium (HIPPC) would be a timely and realistic concept to turn the HUPO-HIPP program described above into deliverable actions using easy-to-track milestones. Initially proposed by Admon and Bassani (Admon and Bassani-Sternberg, 2011), the scope of HIPPC was discussed and included the ambitious goal of mapping the entire repertoire of peptides presented by all HLA allotypes expressed in the human population. It was argued that achieving the vision of a baseline immunopeptidome is important to support the consistent quantitative measurement of immunopeptidomic sample cohorts in the future. Thus, a comprehensive atlas would be generated by a decentralized effort that would include many laboratories around the world to ensure data saturation with the applied technology. Data would be collected from different cell and tissue types under various experimental conditions, in both health and diseases, with a particular focus on cancer, autoimmunity, and infectious diseases. The group also discussed the complexity of the atlas and the current inability to estimate the size of the human immunopeptidome given the extreme diversity of HLA alleles. In fact, data presented by the participants suggested that the immunopeptidome remains hardly predictable and its size is seemingly boundless given the dynamic antigen processing and presentation machinery that constantly reshape the peptide repertoire, the presence of post-translational modifications (e.g., phosphorylation, glycosylation, methylation), the cryptic nature of some peptides, as well as the apparently large fraction of spliced peptides generated by the proteasome. Given the complex nature of the human immunopeptidome, it was suggested that such an endeavor should be a long-term collaborative project potentially supported by several national and international funding agencies such as NIH and EC, as well as philanthropist organizations such as Gates foundation. If successfully funded, the rich source of data generated by HIPPC will be highly beneficial for computational scientists to develop and test new algorithms to understand the rules of antigen presentation and accurately predict T cell epitopes; for basic scientists and clinical researchers to navigate within a large catalog of high-quality context-specific HLA-associated peptides and gain new insights into the composition of the immunopeptidome; and finally, to access HLA allele-specific peptide spectral libraries to facilitate next-generation MS analysis of immunopeptidomes (Caron et al., 2015).

The working group also discussed the potential of establishing strategic partnerships between industry and academia, as well as the impact of sharing large amount of immunopeptidomic data among the HIPPC's participants. In this regard, it was suggested that upon a consensus, large immunopeptidomic datasets generated by the HIPPC's participants would be shared immediately within the consortium for a pre-defined period of time to improve communication among participants and accelerate discoveries. In addition, HIPPC would support the further development of a suitable open immunopeptidomic repository (e.g., SysteMHC Atlas) to facilitate long-term data sharing and interoperability between multiple databases. Notably, data annotation would be standardized and experimental protocols would be taught, though not standardized, as HIPPC would primarily prioritize the development of new experimental and computational approaches. Those new approaches would need to be benchmarked as they become available to the consortium's participants to further guide the development and optimization of immunopeptidomics workflows.

Altogether, it was judged that a new consortium-based research venture is a very timely and realistic project to advance the immunopeptidomics field. As a next step, HIPP will seek support from the HUPO council and will draft a plan in the coming

year to address remaining questions about funding, governance, structure, and coordination. If launched, HIPPC will represent a game-changing initiative to increase the accessibility and impact of immunopeptidomics in immunology, vaccine, and immunotherapy.

Conclusion

MS profiling of HLA-associated peptides will have a profound impact in immunology and precision medicine if further developed and deployed across the community. For 2 days, the HUPO-HIPP working group has discussed what is needed to drive the field forward and make the tools of immunopeptidomics accessible to many scientists. A short-term action plan was drafted and included the launch of a summer school to expand the immunopeptidomics community, the creation of guidelines to standardize data annotation and reporting, the design of multi-laboratory studies to benchmark protocols and MS technologies, the further development of a new public repository for deposition of immunopeptidomic datasets, the necessity of journals' editors to enforce sharing of immunopeptidomic data, and the possible launch of a large-scale human immunopeptidome project consortium, i.e., the HIPPC. If successfully, this community effort will be a major milestone to define the immunopeptidome landscape in the human body, make the tools of immunopeptidomics accessible to many scientists, and importantly, lay the foundation for the development of new immunotherapeutic interventions in precision medicine.

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