HUMAN VACCINES PROJECT

Human Immunomics Initiative
Deciphering Effective Immunity in Aging Populations

Background: Whereas global life expectancy has increased with most people nowadays living into their 60s and many years beyond, these extra years are often not lived in good health [1]. As people age, they not only develop more non-communicable diseases but also become more vulnerable to infectious diseases. The burden of infectious diseases including shingles, bacteraemia, pneumonia, and gastroenteritis, sharply rise above the age of 55-65 [1,2]. Symptoms of infections are also more severe at older age, resulting more often in hospitalization and sequelae that may cause loss of independence. This phenomenon is highlighted in the COVID-19 pandemic with SARS-CoV-2 infections prevailing in older people and more often than in younger population leading to hospitalization and death. Why COVID-19 particularly affects elderly is not yet understood, but an increased expression of ACE2, the receptor for viral entry into the lungs, together with a loss of immune defence mechanisms with progressive aging, are believed to play an important role [3]. Immuno-senescence is the process of the human immune system progressively declining in function with age, and this involves all compartments of the immune system. Aging of the human immune system is associated with increased infection risk, development of inflammatory diseases, and reduced vaccine effectiveness. When COVID-19 vaccines become available, people aged 65 and older will be the first to be vaccinated, together with health care workers. This alone is estimated to require close to 1.5 billion vaccine doses. Experience with other vaccines, however, shows that vaccine effectiveness is lower in older than younger adults and that a different approach may be required to compensate for the loss of function of the aged immune system and increase vaccine-induced protection. In the light of the COVID-19 pandemic, understanding immuno-senescence and its impact on vaccine responses is hence more urgent than ever.

The Human Immunomics Initiative (HII) strives to develop artificial intelligence-powered models of immunity with a specific focus on effective immunity in aging populations. HII is a joint project between the Harvard T.H. Chan School of Public Health and the Human Vaccines Project (HVP). An executive board was established in April 2020, consisting of 5.1.2e 5.1.2e 5.1.2e 5.1.2e

Fundamental to the approach and program of work of HII, is to differentiate between chronological and biological age. Whilst chronological age is an important predictor of morbidity and mortality, it cannot account for heterogeneity in the decline of physiological function and health with advancing age. On the contrary, biological age reflects physiological status and functioning and can account for variation in the trajectory of human aging. Using blood-based biomarkers for liver, kidney, metabolism, immune system, as well as brain function 5.1.2e recently developed an algorithm for "biosystems-age" that was demonstrated to predict risk of mortality and morbidities including stroke, diabetes, cancer, coronary heart disease, and COPD in a cohort of community based individuals aged ≥55 years (Rotterdam Study) (manuscript in preparation). In addition, changes in expression levels of microRNAs (miRNAs) in plasma showed stronger significance with biosystems than chronological age, thereby suggesting that biosystems age is condensing the underlying aging signals.

Immune aging is part of biological aging. Like other systems, changes in immune functioning over lifetime are likely less dictated by chronological age than by individual trajectories that are determined by individual baselines and genetic and environmental interactions. Measuring changes in cell-subset frequencies in healthy adults of different ages followed longitudinally, Alpert et al. found that trajectories of immune aging better described a person's immune status than chronological age and to predict all-cause mortality beyond well-established risk factors [4].

Immune aging involves all compartments of the immune system. Advances in systems biology, bioinformatics, and artificial intelligence now enable to study the immune system and other systems of the human body holistically. HII will take a holistic systems biology approach to develop algorithms for biological age and immune age and to decode the mechanisms and rules of effective immunity at old age.

Scientific Program: Recruitment of study cohorts is time and resource consuming. Instead HII will make use of existing large-scale population cohorts that follow subjects longitudinally for health outcomes and that collect and bio-bank specimen such as blood samples at different moment in time. These cohorts provide access to well-phenotyped populations whilst offering opportunities for additional research. HII has identified three cohorts for its program of work:

1. The first to develop and optimize its research tools (**Lifelines, Netherlands**);
2. The second to develop models of immunity in relation to health status in an aging population (**Rotterdam Study, Netherlands**);
3. The third to recruit subjects for vaccine trials to confirm developed models of effective immunity participants (**Framingham Study, USA**).

HII's first project is to demonstrate technological feasibility of the HII concept in the LifeLines Cohort. This prospective cohort study includes 167,000 inhabitants of the northern provinces of the Netherlands of all ages. The BioSystems-Age model developed by Wu and Goudsmit will be applied to select from each of 8 distinct birth cohorts (i.e. participants aged 15±2 years; 25±2 years; etc. up to 85±2 years at the time of sampling) 100 subjects who are at the extremes of BioSystems-Age, i.e. that have the youngest (50) or oldest (50) biological age for their chronological age. Plasma samples from these 800 subjects will be analysed using the biosystems discussed: IgG glycome, antibody responses to pathogens and self-antigens, proteome, metabolome, cfDNA methylation, and miRNA.

Of the specimens that may be collected in large-scale population cohorts, plasma is the most collected and stored specimen available for additional research. HII will therefore use plasma from the three above-mentioned cohorts to take a biosystems approach to phenotype status and functioning of various physiological systems. This will include: profiling of antibodies against pathogens and self-antigens; IgG glycosylation; proteomics; metabolomics; miRNA; and cfDNA methylation.

Humoral responses to infection can persist over years or decades. Certain infections such as measles may eliminate memory B cells against other pathogens [5]. The immune system may also produce antibodies against self-antigens that can result in auto-immune diseases. "VirScan" is a high-throughput method based on phage-immunoprecipitation-sequencing (PhIP-Seq) that was developed to allow a comprehensive analysis of antiviral antibodies in human sera [6]. An adapted bacteriophage library now includes peptide epitopes from major human bacteria and self-antigens in addition to viruses[7,8]. Comprehensive profiling of plasma antibodies against more than 400 viruses, bacteria and self-antigens will provide insight into immunological footprint and possible ability to respond to new pathogen encounters and vaccines.

Antibody effector function is determined by the constant (Fc) region. This involves binding of antibody isotypes and subclasses to Fc receptors (FcR) differentially activating functional responses. This is further influenced by antibody glycosylation. Variation in galactosylation, fucosylation and sialylation of IgG can result in 24 different IgG glycan structures. Kristic et al. defined an algorithm for 'GlycanAge' based on the IgG glycome that was independently associated with chronological and biological ages [9]. Taking a similar high-throughput approach, glycosylation of plasma IgG will be studied.

Plasma contains proteins from nearly every cell and tissue in the body. Proteomic changes in plasma may hence reflect aging of different cell types and tissues [10,11]. In addition, metabolites are unique chemical fingerprints of specific cellular processes and signatures of the plasma metabolome have been associated with biological age and rate of aging [12]. Proteomics and metabolomics will hence be performed to study the role of components of the plasma proteome and metabolome in biological and immune aging.

The epigenome represents chemical changes to DNA and histone proteins that affect the functional use and stability of DNA and as such connect the genotype with the phenotype. Epigenetic changes can be spontaneous or driven by external or internal influences and may explain differences in aging against a similar genetic background. Methylation of DNA occurs at **CpG sites** near the promoters of genes. The state of methylation of CpG islands is critical to both gene activity and gene expression. In an earlier feasibility study a high overlap between CpG on plasma cell free DNA (cfDNA) and genomic DNA (gDNA) from PBMC was demonstrated. Methylation of cfDNA in plasma will therefore be quantified as a read-out of the 'epigenetic clock'.

In summary, over the next 2-3 years, the initial studies from samples of the Lifelines cohort will optimize methods for developing models of effective immunity in aging populations which then will be assessed in the Rotterdam Study, and then validated in vaccine trials in the Framingham Study.

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