Human immune system variation

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Abstract | The human immune system is highly variable between individuals but relatively stable over time within a given person. Recent conceptual and technological advances have enabled systems immunology analyses, which reveal the composition of immune cells and proteins in populations of healthy individuals. The range of variation and some specific influences that shape an individual's immune system is now becoming clearer. Human immune systems vary as a consequence of heritable and non-heritable influences, but symbiotic and pathogenic microbes and other non-heritable influences explain most of this variation. Understanding when and how such influences shape the human immune system is key for defining metrics of immunological health and understanding the risk of immune-mediated and infectious diseases.

The immune system is intrinsic to health, but translating what we have learned about basic immunology from animal models to humans has been a major challenge, with many more failures than successes[1–3]. To improve our knowledge of the human immune system, immunologists are now looking at different ways to directly investigate the immune status of humans[3–5]. There has been a pressing need for new research strategies that could work within the constraints of humans, as many of the manipulations that are standard in mouse immunology cannot be directly translated to humans. One of the most promising strategies is adapted from systems biology and is referred to as systems vaccinology[6] or systems immunology[3]. In general, systems biology approaches seek to identify the major components of a given system and measure how these components change in response to perturbations of the system. In the immune system, the main components are the different types of immune cells and the cytokines that they communicate with. Fortunately, the majority of these components can be measured with available technologies and a representation of these components is present in a blood sample — which is widely available in human studies.

A wide range of factors can perturb the human immune system, but the most convenient to investigate for systems immunology is the response to standard vaccinations such as influenza virus vaccines and, in particular, the very effective and robust yellow fever vaccine. Systems vaccinology can reveal which components of the immune system change and how they change in response to perturbations, and this in turn yields information about the sensitivities of a given person's immune system and the variation of immune responses between individuals. This information might predict responsiveness or non-responsiveness to vaccines, which is an important problem for less robust vaccines, such as the influenza vaccines, and especially when administered to very young or elderly individuals. By focusing mainly on blood, a systems immunology approach can be informative about both healthy and ill individuals, as well as young and old. In addition, systems approaches make use of the fact that specialized cells in the immune system are both the detectors and effectors of the immune system, that these cells communicate with each other through cytokines and direct interactions and that a global representation of what is happening in the immune system of a particular person at a given time can be estimated by analysing such interactions. Although blood is not an immunological organ per se, it is the conduit for most immune cells circulating in the body, especially after an immunological stimulus such as vaccination (FIG. 1). As an illustration of this, Wilson and colleagues found that 50–80% of circulating plasmablasts were specific for antigens in the vaccine seven days after an influenza virus vaccination[7]. A similar time course has been shown for gluten-specific $CD4^+$ T cells following gluten challenge in patients with coeliac disease[8,9].

The recent development of many new high-throughput technologies enables simultaneous measurements of many cell types, cytokines and other biomarkers of immune function in the same blood sample. Such advances provide an opportunity for studying human immune system variation at a global scale, taking co-variation of specific cell populations and proteins into account. Recent population studies have also showed that human immune system variation can now be studied globally, and the influences of age, sex and specific environmental factors can be addressed. These studies are timely and complementary to the many studies investigating genetic influences on immune system function and immunological diseases.

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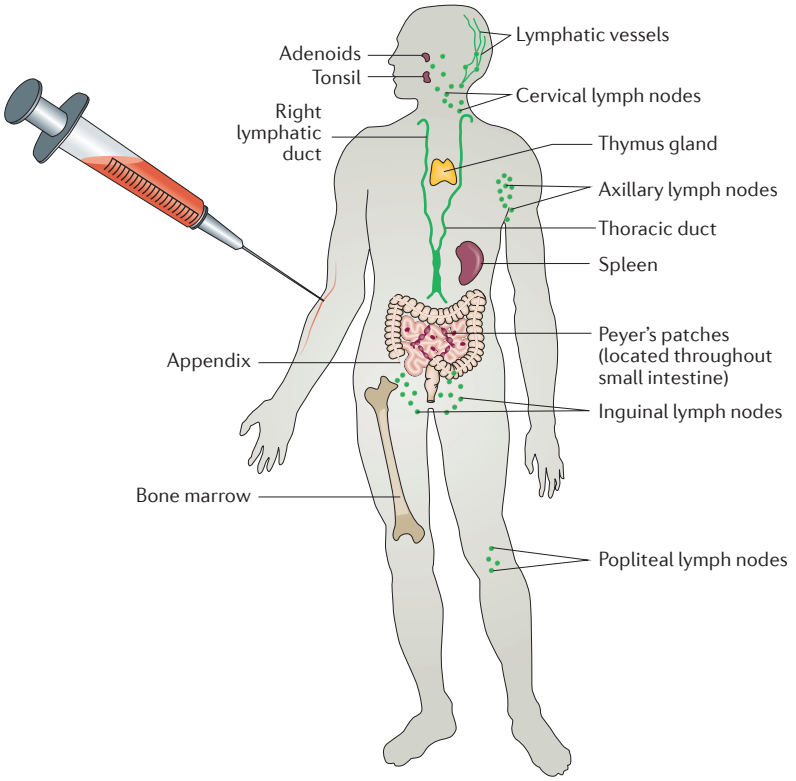

Figure 1 | **The blood as a window for global immune system analysis in humans.** Although the blood is not an immunological organ per se, it is the conduit for most immune cells circulating in the body, especially after an immunological stimulus such as vaccination, allowing even distal processes to be reflected in a blood sample that is readily accessible even in humans.

A combined understanding of both the heritable and the non-heritable influences on immunity is necessary to fully understand inter-individual variation and its consequences on immunological health and disease. The immune system varies between different tissues within an organism, but in this Review we focus on peripheral blood since it is the most well characterized tissue in these early days of systems immunology. We focus on our current understanding of human immune system variation within individuals over time and between individuals in different age groups and of different sex, and we discuss the specific environmental exposures that shape human immune systems.

Technological advances

There have been a number of important advances in technologies that enable high-dimensional immune system analyses (BOX 1). The possibility to analyse many, if not all, immune system components in the blood allows novel questions to be answered, specifically relating to the interactions between the many components of human immune systems[10]. Such approaches are providing novel understanding of immune system regulation in health and disease[11]. Also, by globally profiling, for example all mRNA transcripts, unexpected pathways activated under a specific condition, such as after vaccination can be revealed[12–14].

Time-dependent immune system variation

Variation over time within individuals. When studying the immune response in an individual during the course of an acute infection or some other perturbation, such as during a vaccine-induced immune response, the immune system would seem to be a continuously moving target. However, this is not the case outside of such episodes, at least not in healthy adults. Blood samples taken weeks to months apart from healthy adults show very stable immune cell frequencies and serum protein levels[15,16]. Our own analyses over the course of yearly samplings suggest that immune profiles remain stable even at longer intervals (up to 6 years) in healthy individuals[17]. This suggests that each individual has a baseline state of immune system composition in which cells and proteins are well regulated, and the balances between these are optimal for the current conditions (FIG. 2a). Immune responses to an acute challenge leads to drastic changes that involve expanding cell populations and stark increases in serum protein concentrations, which will quickly return to the same baseline state as before the challenge (FIG. 2a). The mechanisms that regulate such systems-level coordination and regulation are poorly understood but have become amenable for study in recent years thanks to the technological developments that enable simultaneous measurements of all system components in the same sample, and these analysis will help to improve our mechanistic understanding.

Immune system variation with age. Young children and elderly individuals are more susceptible to infections than other age groups[18,19]. The infant has an immune system biased towards tolerance as a consequence of life *in utero*, and it consists of cells with mostly naive phenotypes that mature when exposed to the environment. The neonatal immune system depends on different protective cell populations compared with adults[20], and qualitative differences in immune responses by shared cell populations between young children and adults have also been reported[18,21]. More work is needed to fully appreciate all the differences between the immune systems of children and adults, with the potential for improved vaccination strategies in the future[22]. The immune systems of very old individuals are characterized by loss of immune cells, lymphopenia and reduced diversity of variable receptor genes on B cells[23] and T cells[24], although this reduction in T cell diversity seems less pronounced than previously thought[25]. Still, it is possible that changes in relative frequencies of specific adaptive lymphocyte clones that differ in phenotype, could contribute to some of the changes in the immune system composition with advancing age. It is important to note that even if specific parameters are found to correlate positively or negatively with age, this cannot be taken as proof of their involvement in the process of ageing. Environmental factors can often influence individuals differently during different stages of life and adaptive changes in the immune system to such factors could explain age-correlated immune system parameters.

Increased concentrations of pro-inflammatory cytokines such as tumour necrosis factor (TNF) have been found in the circulation of some elderly individuals,

Box 1 | **Details of high-dimensional immune system analyses technologies**

Immune cell analyses

Flow cytometry. This technique enables single cell analysis by using fluorescently labelled antibodies measuring up to 30 simultaneous parameters in millions of individual cells in the most technically specialized laboratories[98]; however, most flow cytometric protocols detect 15 or fewer simultaneous parameters. The speed and versatility of the technology and the ability to sort viable cells makes flow cytometry a cornerstone technology in immunology research.

Mass cytometry. Single-cell analysis using antibodies tagged with unique mass-reporters are detectable at single-cell resolution using an inductively-coupled plasma mass spectrometer (ICP-MS) system[99]. Cytometry-by-time-of-flight (CyTOF; Fluidigm Inc) readily allows the simultaneous measurement of approximately 45 parameters, including proteins and nucleic acids[100] in millions of individual immune cells, which enables a unique combination of width and depth of analysis into the cellular immune system[37]. Both phenotypic and functional measurements such as cytokines[101] and intracellular signalling[102,103] can be addressed simultaneously, which enables assessment of both phenotypes and functions.

Single-cell gene expression analyses. Sequencing analysis methods in single cells have developed rapidly in recent years. Currently, global transcriptome analyses in several thousands of individual immune cells are possible using the latest protocols, allowing analyses of gene-regulatory patterns in such cell populations and refined atlases of cell populations[104]. Gene expression analyses also provide the attractive possibility of analysing variable receptor genes, such as those encoding T cell and B cell receptors to determine the clonality of immune cells and their specificity, and combining such information with simultaneous analyses of functional properties. Both PCR-based[105] and sequencing-based methodologies[106] have been developed for such analyses.

Analyses of serum proteins

Bead array methods using fluorescent bead readouts are popular and commonly used for analysis of serum proteins. Another approach with sensitive detection due to dual recognition of proteins is offered by proximity-extension assays (ProSeek; Olink AB)[38], in which affinity reagents are detected by associated nucleic acid probes. Also, mass spectrometry-based plasma proteomics have re-emerged in recent years owing to the developments in fractionation methods, instrumentation and analytical approaches, which enable the broadest analysis of the ~3,000 plasma proteins that are present at variable concentration in humans[107].

which suggests there is a low-grade inflammatory state in these individuals[26]. A recent population analysis of over 1,000 individuals found that 24 out of 92 protein biomarkers in the serum of adults were strongly influenced by age[27]. Vaccine responses are poor in some older individuals, and baseline biomarkers that can predict poor responsiveness are beginning to emerge[15,28,29]. Although much work is needed to better understand the overall changes in immune system composition and function over the course of life, it is clear that age is an important factor to consider when assessing human immune variation.

Seasonal and circadian immune system variation. The incidence of autoimmune type 1 diabetes in children varies over the seasons, with lowest incidence in the summer months and highest incidence in the autumn and winter in the northern hemisphere[30]. Many patients with rheumatoid arthritis subjectively experience seasonal variation in joint symptoms, and one study performed in Japan found evidence of such seasonal variation in disease severity scores[31]. An analysis of blood gene expression profiles has revealed clear seasonal patterns, but the differences primarily involved genes expressed in platelets and red blood cells, the frequencies of which are known to vary seasonally[32]. Another study of gene expression patterns across multiple cohorts identified seasonal changes in the expression of genes thought to be unique to specific immune cell subpopulations, which suggests changes in the immune cell composition over the course of the year[33]. This finding needs to be confirmed by more direct measurements of immune cell frequencies over time.

Circadian variation in inflammatory manifestations, such as stiffness and pain being worst in the morning hours, is a defining symptom of several autoimmune conditions including rheumatoid arthritis[34]. This variation has been attributed to concomitant circadian regulation of endogenous hormones such as cortisol. In patients with rheumatoid arthritis, worsening of the symptoms has been shown to coincide with a spike in serum levels of interleukin-6 (IL-6) early in the morning[35]. In mice, antimicrobial responses have been shown to differ during certain times of the day; hence a pathogen encounters different immune responses in the daytime and at night[36].

Inter-individual variation

It is widely accepted that human immune systems are variable between individuals, but the extent of variation is only starting to become clear. Recent advances in cytometry[37] and multiplex serum protein measurements[38,39] enable simultaneous analyses of the cells and proteins that constitute human immune systems. These analyses enable estimates of inter-individual variation, not only at the level of individual measurements but also at the systems-level across hundreds of individuals within a population. By considering inter-dependencies between these immune system components, we can also learn how these measurements co-vary in health and disease.

Quantifying inter-individual variation. Several cohort studies have analysed immune cell frequencies and serum protein concentrations in healthy adults in recent years[11,15,16,40]. Here we use data from two separate cohorts of healthy individuals recruited and sampled at the Clinical and Translational Research Unit at Stanford University Hospital, USA, and immune cell frequency measurements made using mass cytometry at the Human Immune Monitoring Center also at Stanford University[11,17]. Using this data, we illustrate the range of variation observed in the relative frequencies of six principal immune cell populations: B cells, monocytes, natural killer (NK) cells, $CD4^+$ T cells, $CD8^+$ T cells and total T cells (FIG. 2b). The range of variation among these healthy individuals is many orders of magnitude and individuals completely devoid of specific cell populations such as monocytes ($CD33^+$ cells) and NK cells ($CD3^-CD56^+$ cells) were identified. The frequency of $CD4^+$ T cells as a fraction of the total T cell population ranges between 22–90% and the fraction of $CD8^+$ T cells ranges between 6–65% (FIG. 2b). The B cell fraction ranges between 4–69% of the total number of lymphocytes (FIG. 2b). The fact that seemingly healthy individuals display such a large degree of variation in specific immune system components suggests novel avenues for future studies into the mechanisms ensuring robustness

Rheumatoid arthritis
An immunological disorder that is characterized by symmetrical polyarthritis, often progressing to crippling deformation after years of synovitis. It is associated with systemic immune activation, with acute-phase reactants being present in the peripheral blood, as well as rheumatoid factor (immunoglobulins specific for IgG), which forms immune complexes that are deposited in many tissues.

Cortisol
A steroid hormone produced by the adrenal glands and released in response to stress and has a generally suppressive function on the immune system.

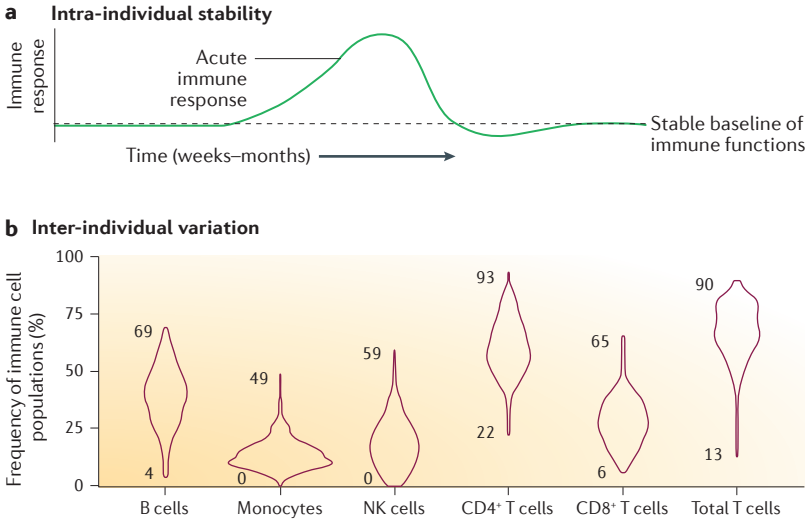

Figure 2 | **Variation in immune cells and proteins. a** | An illustration of the observed stability of most immune cell and protein measurements over the course of weeks to months. During acute immune responses drastic changes occur, but thereafter measurements seem to return to a stable baseline. **b** | Distributions of six principal immune cell populations from a Stanford cohort ($n = 398$) of healthy adults[11,17]. Numbers indicate minimum and maximal values observed.

and redundancy in the immune system. A complex system, such as the immune system, probably uses adaptive strategies, compensatory pathways and functional redundancy to maintain its vital functions even in 'outlier' individuals.

The structure of immune system variation in human populations. When analysing an increasing number of individuals with respect to the composition of immune cells and proteins in their immune systems, it is important not to catalogue only the range of variation for individual measurements, but also to investigate novel associations between immune system components and the structure of variation between individuals. If the composition of cells and proteins that make up an individual's immune system is referred to as the individual's 'immunotype', it is interesting to study whether such immunotypes are distributed as discrete groups or as a continuum (FIG. 3). Such a global understanding of human immune system variation could help identify individuals with outlier immunotypes and immunotypes associated with increased risk of severe infections or immune-mediated disease. Many studies have used global gene expression profiles to define variation between patients, for example patients with systemic lupus erythematosus (SLE) can be grouped into seven discrete groups of patients based on global gene expression profiles and disease severity[41]. SLE is a disease notorious for its heterogeneous clinical presentation, and it is unknown whether such discrete groups can be defined also in healthy individuals.

Immune system variation by sex. Many immune-mediated disorders show different incidence rates between men and women — for example, 80% or more of the patients with autoimmune diseases, such as Sjogren syndrome, SLE and autoimmune thyroid disease, are women[42], whereas the incidence and severity of ankylosing spondylitis is higher in men[43]. Differences in baseline states of immune cell frequencies, serum protein concentrations and functional properties between men and women are not well defined[16]. In a study of 1,005 Swedish individuals, a few serum proteins such as E-selectin, growth hormone, fatty acid-binding protein 4 and tartrate-resistant acid phosphatase type 5, differed significantly between females and men at the baseline state, but the consequences of these differences on immunity are unclear[27]. Gene expression analysis of whole blood samples has indicated clear differences between men and women, both for autosomal genes and for genes expressed on X and Y chromosomes[44]. Our current understanding of differences in baseline immune profiles between men and women is incomplete, but taking sex into account when analysing immune system variation is important, and if sex is reported in publications together with immunological data, the situation can become clearer in the future. The influence of sex on functional immune responses, such as the response to vaccination, is also unclear but is under investigation. It is often thought that women typically mount stronger immune responses than men owing to the immunomodulatory effects of oestrogen as an enhancer[45], and testosterone as a suppressor, of humoral immunity[46]. Alternatively, such sex differences could be explained by differences in the kinetics of the immune responses, with men showing a peak in immune responses at day 1 after challenge, whereas women elicit their peak responses at day 3 post challenge as suggested by one recent analysis of gene expression responses to influenza virus vaccination[13]. As data from multiple studies are being reported the, often subtle, effects of age and sex on immune cell and protein profiles will become clearer and we should have sufficient statistical power to disentangle their effects on human immune system variation. Public repositories for immunological data, such as the ImmPort database[47], will be useful in this respect by allowing for meta-analyses across studies and cohorts.

Heritable influences

The manifestation of infection in individual patients is known to be influenced by host genetics[48,49], with severe infections occurring in childhood often representing a monogenic immunodeficiency and severe manifestation during secondary infection being a result of more complex genetic predisposition[50]. The contribution of heritable and non-heritable factors to the composition and function of specific immune system components is less clear. Many studies have been performed exploring possible heritable traits associated with specific immune system measurements. Typically, genome-wide association studies (GWAS) are designed to associate genetic loci with individual immune system measurements, such as specific immune cell frequencies or the concentration of a specific cytokine. A separate line of investigation focuses on associating genetic loci with the occurrence of immune-mediated diseases and provides genetic leads for further aetiological studies. In particular the ~80 autoimmune conditions affecting humans have been

Systemic lupus erythematosus
(SLE). An autoimmune disease in which autoantibodies that are specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, especially in the kidney. Patients with SLE generally have abnormal B cell and T cell functions.

Sjogren syndrome
A long-term autoimmune disease affecting mucous membranes and moisture-secreting glands of the eyes and mouth, resulting in decreased production of tears and saliva, but there are also systemic manifestations such as muscle and joint pain and fatigue.

Ankylosing spondylitis
A long-term inflammatory disease, more common in men than women, affecting the joints of the spine causing vertebrae to fuse together.

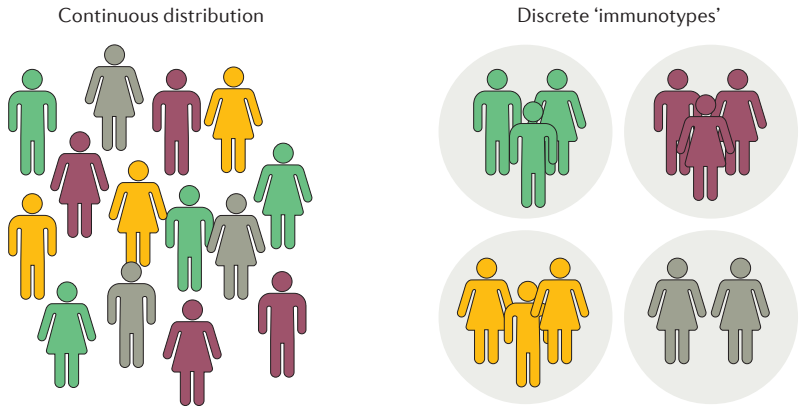

Figure 3 | **Distribution of immune system variation in human populations.** There are two possibilities for human immune system variation, either individuals are distributed continuously with respect to their immune system composition or in discrete groups, so called 'immunotypes'.

extensively studied, and many genetic risk variants have been found[51,52]. Here we focus our discussion on the heritable influences explaining the inter-individual variation of immune system components in the blood, such as frequencies of immune cell populations and serum protein concentrations. We also discuss some analyses performed to discern heritable influences on immune cell functions.

The genetics of immune cell frequencies. White blood cell (WBC) counts are key diagnostic measurements because of their sharp increase during acute infections. Several population studies have found a moderate heritability of WBCs of about 0.38 (REF. 53), and specific loci that could partially explain the variation between individuals have been identified[54]. Also, the total frequencies of lymphocytes, monocytes, neutrophils, eosinophils and basophils are moderately heritable from 0.14 for basophils to 0.4 for monocytes[53]. Some of the specific loci that regulate immune cell frequencies have also been associated with immune-mediated disorders, such as a locus on chromosome 2 containing *ITGA4*, which is associated with monocyte counts[54] and coeliac disease[55]. Using a slightly different approach, two studies analysed smaller populations but measured many more concurrent immune cell population frequencies by high-dimensional flow cytometry, which revealed an additional 24 loci associated with >20 different immune cell populations[40,56]. Together these studies clearly show that a fraction of the total inter-individual variation in immune cell frequencies can be explained by specific genetic variants.

The genetics of serum protein concentrations. Many immune-mediated disorders are characterized by dysregulated cytokine profiles, for example SLE[57,58], which has a gene expression signature dominated by interferon-inducible genes in the blood[59]. The key pathogenic cytokine interferon-α (IFNα) in SLE is increased in the serum of patients with SLE but also in their healthy first-degree relatives, which suggests genetic influences on the IFNα serum levels[60]. Furthermore, the concentration of other cytokines can be influenced by genetic variants, such as the levels of IL-18 in older individuals[61]. Several additional genetic associations have been made between genetic variants within cytokine genes and immune mediated diseases, but whether such genetic variants actually contribute to the variation in serum concentration of the cytokine itself is often difficult to determine[62]. In our own analyses of twins, we found that serum cytokines and chemokines ranged from 0 to 1 in estimated heritability, but with an average heritability slightly higher than what was found for immune cell frequencies[11]. This finding could be explained by the fact that cell frequencies might be regulated by more complex and polygenic influences, whereas serum proteins are the direct products of individual genes.

Heritable influences on functional immune responses. The ImmVar project is a cohort study involving individuals of African-American, East Asian and European ancestry in the Boston metropolitan area, and within this project detailed analyses were performed to investigate variability in functional responses between individuals, specifically responses by T cells and dendritic cells (DCs). Gene expression profiling of these cells revealed that 22% of the overall variation in gene expression between individuals could be attributed to heritable factors[63]. This is in line with previous analyses showing a minor to moderate contribution of heritable factors on the inter-individual variation of blood transcriptomes[64,65]. Additional reports from the ImmVar cohort revealed substantial inter-individual variation of gene expression profiles, with minor to moderate heritable influence on gene expression patterns in DCs upon pathogen sensing[66] and in $CD4^+$ T cells after activation of T cell receptor signalling *in vitro*[67]. Interestingly, several of the identified variants have been previously associated with immune-mediated diseases[66,67]. In a twin study, analysis of cytokine-induced signalling responses across multiple immune cell populations was shown to be highly variable between individuals[11]. Most of these responses, which were induced by cytokines like IL-6, IL-10, IFNα and IFNγ, showed very limited heritability. By contrast, the phosphorylation of signal transducer and activator of transcription 5 (STAT5) in both $CD4^+$ and $CD8^+$ T cells after stimulation with homeostatic cytokines IL-2 and IL-7 was almost completely explained by heritable factors[11]. Together these results show important differences in the heritable and non-heritable influences that regulate different functional properties of human immune systems.

Non-heritable influences

The immune system is a sensory system for internal and external stimuli. Similar to other sensory systems, the cells of the immune system must adapt to inputs received in order to maintain its responsiveness to relative, rather than absolute, changes in stimuli over time[68,69]. It is therefore conceivable that adaptive changes induced by environmental influences would be important in shaping the composition and function of an individual's immune

system. Non-heritable influences are typically interpreted as environmental influences, such as infections and vaccines, but should rather be considered a common denominator for all relevant influences that do not have germline inheritance, including *de novo* mutations and stochastic epigenetic changes, in addition to the influences exerted by pathogenic and symbiotic microorganisms. Such stochastic epigenetic changes are interesting and poorly understood, but they occur in immune cells with every cell division, owing to the imperfect fidelity of the replication machinery, and have the potential for real influence on immune cell phenotypes. Stochastic changes can give rise to globally different epigenetic patterns between monozygotic twins during a lifetime[70]. However, given that epigenetic changes can also be induced by environmental stimuli, distinguishing cause and effects for epigenetic changes observed in immune cells is very difficult. To improve such studies, better study design focusing on interventions and longitudinal profiling is needed[71].

Influences of the microbiota. In mice the contribution of the microbiota, similar to the influence of other environmental factors, can be investigated in isolation owing to the controlled environments in animal facilities. The use of germ-free mice has indicated several important effects of the microbiota on the mouse immune system, even at the level of specific strains of bacteria. For example, normal development of lymphoid tissues in the gut depend on interactions with gut bacteria, and several immune cell populations show numerical and functional deficiencies in germ-free mice[72]. The effect of the microbiota seems to be species specific, which suggests co-evolution between specific bacterial strains and their hosts[73]. The controlled environment in animal facilities represents both an opportunity and a curse — specifically when trying to understand normal microbiota–immune system interactions. Recent studies have found that wild mice or pet-shop mice, which have a more natural pathogenic exposure history, exhibited immune system profiles that were more comparable to human immune systems than normal laboratory mice[74]. Furthermore, when mice are infected with common pathogens before vaccination it induces altered gene expression in response to the vaccine and this is comparable to human vaccine responses[75]. These findings illustrate the difficulty in translating findings made in germ-free and specific-pathogen-free mice housed in clean facilities to humans[3].

The microbiota has an important role in shaping the human immune system. In 1989, the hygiene hypothesis was proposed by Strachan to explain epidemiological data showing an increased incidence of in immune-mediated conditions such as hay-fever, asthma and eczema coinciding with increased hygiene in the post-industrial society[76]. Also, autoimmune conditions such as type 1 diabetes, multiple sclerosis and Crohn disease are thought to share some of these mechanisms of immune perturbation as a consequence of improved hygiene and the ensuing reduction in pathogens encountered early in life[77]. Strong evidence supports a protective effect of early-life exposure to farm environments and, in particular, support a role of endotoxin in inducing tolerance to common environmental antigens[78]. In fact, exposures to different strains of bacteria carrying different versions of endotoxin can exert different influences on developing immune systems, which could possibly explain some of the striking differences in incidence of immune-mediated diseases observed between different populations[79].

Bacterial dysbiosis and its effect on human immune systems. Apart from the effect of endotoxin, microbial dysbiosis — which is defined as an imbalance between specific species in the colonizing microbiome — has been linked to an increased risk of asthma, suggesting an immune system-perturbation during the first 100 days of life in humans[80]. More locally in the gut, the intestinal microbiota has been linked to initiating and maintaining inflammation as well as determining the presentation of inflammatory bowel diseases such as Crohn disease and ulcerative colitis[81]. This crosstalk between immune cells and microorganisms in the gut is also illustrated in patients undergoing allogeneic stem cell transplantation and suffering from intestinal graft–versus–host disease (GVHD). The inflammation induced by alloreactive T cells during GVHD seems to give rise to a dysbiosis among gut microorganisms, which can influence the duration and severity of the inflammatory response[82]. Although the effects of the microbiota on intestinal immune responses can seem obvious, perhaps a more surprising finding is the link between the microbiota and the humoral immune response to non-adjuvanted vaccines. For example, Toll-like receptor 5 (TLR5) — which mediates sensing of flagellin on bacteria — is necessary for optimal plasma cell activation and antibody production in response to vaccination[83]. Thus, inter-individual variation in vaccine responses could be influenced by differences in the gut microbiota[83]. Furthermore, the gut microbiota can influence the microenvironment surrounding tumours, which has implications for the responsiveness to chemotherapy[84] and immune-modulatory agents[85,86].

The influence of viruses on the human immune system. Humans have co-evolved with viruses for millennia, during which some viruses have integrated into our genomes whereas others have established life-long chronic infections. Broad serological profiling has revealed that at any given time, an individual carries antibodies to about 10 different viral species[87]. Several viruses such as the cytomegalovirus (CMV) have been extensively studied as modulators of host immune systems. CMV is thought to reactivate regularly after the primary infection and each time it reactivates it induces changes in the host immune system such that about 10% of the T cell repertoire is CMV specific[88], and other cell types, such as NK cells[89], also adapt their phenotypes to the presence of CMV. An analysis of monozygotic twins discordant for CMV showed that 119 out of 204 immune cell frequencies and serum proteins had a reduced twin–twin correlation compared with CMV-negative monozygotic twins, which suggests that this virus broadly influences

Hygiene hypothesis
A hypothesis stating that the lack of early childhood exposure to infectious and symbiotic microorganisms increases the susceptibility to allergic diseases later in life, by altering the normal development of the immune system.

Graft–versus–host disease
(GVHD). An immune response mediated by donor T cells contained in a transplanted allograft and directed against the recipient. GVHD is not associated with solid-organ transplantation but can occur with bone marrow or haematopoietic stem cell transplants.

the composition of an individual's immune system[11]. In another analysis, the presence of CMV in younger adults (20–30 years of age) was associated with stronger immune responses to the flu-vaccine in healthy individuals, suggesting beneficial effects of CMV infection for immunocompetent individuals[90]. Humans are constantly reinfected by low-virulence viruses that can induce immune responses and probably also adaptive changes in cell frequencies and functions, which can shape an individual's immune system and influence the risk of immune mediated disease[91]. Furthermore, the interactions between viruses and host immune cells have been supported by an analysis of dynamical changes in the blood virome (by sequencing cell-free DNA) of patients who had undergone organ transplantation and were treated with immunosuppressive drugs. Many viruses, for example members of the Anelloviridae family, varied with the extent of immunosuppression and clinical outcome, which illustrates a relationship between these viruses and host immune competence[92]. Although it is important to consider the presence of common viruses such as CMV when assessing human immune variation, an individual's immune system is shaped by the complete viral history and this should be taken into account.

Non-microbial environmental factors. Humans live in a complex environment, and although the influences of microorganisms in shaping human immune systems are the most well-described factors, many other environmental factors can influence our immune systems. Cigarette smoke and its ~4,500 components exert broad and damaging effects both on local immune parameters in the lung and systemically[93]. For example, current smokers have increased total leukocyte counts, a phenomenon that is reversible upon smoking cessation[94]. Smokers also have reduced overall levels of serum immunoglobulins and reduced NK-cell functional activity[93,95]. The specificities of antibodies are also altered in smokers with a higher general abundance of autoantibodies[96] and antibodies specific to post-translational modified peptides, such as citrullinated peptides, that are of clinical importance in autoimmune diseases such as rheumatoid arthritis[97].

Conclusion

With the advent of systems analyses of human immunity, we can broadly assess human immune system variation in increasing numbers of individuals and consider interdependencies between immune system components and analyse their variation between individuals in health and disease. So far most studies have been performed in blood, but as we expand the use of systems immunology analyses we can assess the global structure of variation in human populations and this will have implications for the understanding of immunological health and prediction of disease risk. In cancer research, the success of the new immunological therapies has inspired a surge in treatments aiming to modulate immune systems for the treatment of cancers and such developments will benefit greatly from a better general understanding of human immune system variation and the mechanisms underlying this variation. The idea of personalized therapy or precision medicine stems from the realization that individual patients vary with respect to their disease mechanisms and requirements for successful treatment and by determining what these requirements are for the individual patient, better outcomes can be achieved. Here, the issue of human immune variation, both during health and disease will be essential to take into account.

We also believe that better understanding of the mechanisms by which individuals' immune systems vary might help to develop therapies that target such mechanisms to modulate the immune response, either to alleviate an immune mediated disorder, such as chronic inflammatory disease or allergy, or to potentiate a desired immune response against vaccines, pathogens or tumours. In the more long-term perspective, understanding when and how an individual's stable immune system state is established might help us promote the long-term immunological health for all populations through the optimization of modifiable environmental conditions. More generally, systems immunology will also help us understand the immune system as a whole, not just in the fragments that are typical of modern biology. This is likely to reveal novel interactions and lead to more effective modelling of immune function and dysfunction than is currently possible.

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Competing interests statement
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DATABASES
ImmPort: https://immport.niaid.nih.gov/

FURTHER INFORMATION
Interactive twin data visualization: http://brodinlab.com/twins.html

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