Shaping variation in the human immune system

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Immune responses demonstrate a high-level of intra-species variation, compensating for the specialization capacity of pathogens. The recent advent of high-depth immune phenotyping projects in large-scale cohorts has allowed a first look into the factors that shape the inter-individual diversity of the human immune system. Genetic approaches have identified genetic diversity as drivers of 20-40% of the variation between the immune systems of individuals. The remaining 60-80% is shaped by intrinsic factors, with age being the predominant factor, and environmental influences, with cohabitation and chronic viral infections identified as key mediators. Here we review and integrate the recent high-depth large-scale studies on human immune diversity, with its potential impact on health.

Dissecting Variation Through Population Immunology Studies

The evolutionary arms-race between pathogens and hosts includes the constant refinement of host anti-pathogen mechanisms, and the reciprocal development of microbial strategies to circumvent these defenses [1]. While multi-cellular hosts bear the advantage of being able to specialize cells (our immune system) to the task of pathogen clearance, micro-organisms have the undoubted advantage in evolutionary speed. Because of this, the inter-individual diversity of the immune system in a species is an important mechanism for limiting the impact specific pathogens can have on the morbidity and mortality of a population. The maintenance of inter-individual diversity within the immune system is therefore a critical aspect of its function, albeit one that is often neglected in immunological research, for example, through the use of genetically identical mice housed under specific pathogen free conditions [3].

The archetype of diversity generation in the human immune system is the production of extra-genomic variation in the antigen receptors of the adaptive immune system (T cell receptor and B cell receptor). The combination of permutational choice in gene fragment selection with an error-prone system creates an essentially personal T cell and B cell receptor repertoire for each individual, with response capacity further molded by the structural diversity encoded by the highly polymorphic MHC locus [4, 5]. Clonal selection further shapes the repertoire in the periphery. However, even beyond the process of adaptive diversity, an enormous amount of variation exists in the number and functional status of each immunological component. Leukocytes, one of the key cellular mediators of the immune system, are epigenetically modified by the micro-environment and a myriad of other interactions to the point where each individual cell could be considered to exist in a unique status [6, 7]. These interactions shape the composition of an individual's immune system over their lifespan, for example driving the differentiation of naïve T cells into subsets with specialized functions, resulting in the context-dependent generation of Th1, Th2, Th17, follicular helper and regulatory subsets [8].

While a major focus of immunology research over the past decades has been in identifying and functionally dissecting these alternative activation states, there has been a growing appreciation that the plasticity of the immune system and the plethora of subsets and activation statuses results in dramatic individual-to-individual

variation in the immune system. In this review we concentrate on population immunology (see Glossary) studies, which we define as research which directly compares the status of the immune system (relative number of leukocyte subsets, activation status of leukocytes, production of non-cellular mediators) across a large number of individuals for the purpose of identifying the drivers of variation in the human immune system.

Human Immune Variation

The human immune system has a high degree of variation present between individuals. This variation manifests at the cellular and intra-cellular level, such as the differences in the relative frequency of different leukocyte populations and subsets, variation in the transcriptional and translational profiles of leukocyte subsets and variation in the functional capacity and polarization in response to immunological challenges such as vaccines [9]. This variability results in individuals with a different intrinsic susceptibility to different diseases. As an example, the concept of individuals being “Th1-biased” or “Th2-biased” is an articulation of this intrinsic variability, albeit a simplification of the multiple potential states the human immune system could adopt. More broadly, we consider the constellation of multiple cellular and molecular immune parameters within an individual to constitute the immune phenotype of that individual.

The use of high detail longitudinal analysis of the human immune system has revealed this immune phenotype to be highly stable. In principle, the variation present in immune phenotypes within the human population could be generated through either of two mechanisms. First, the human immune system could undergo a continual flux within each individual (producing high levels of intra-individual variation), a process which would in turn produce a high degree of inter-individual variation (**Figure 1a, Online Video 1**). Alternatively, the immune profile of individuals could have relatively low levels of temporal variation in a given individual, but with highly divergent phenotypes between individuals (**Figure 1b, Online Video 2**). In this case, an individual immune phenotype would constitute a ‘stability island’, around which the immune parameters remain fixed over time. Multiple immune phenotyping studies, investigating the relative frequency of leukocyte subsets and activation states, have found that while inter-individual variation is high, the immune profile of

each individual is remarkable stable, even over the course of multiple months [10-13], favoring the second mechanism described above.

The long-term stability of an individual's immune system in the absence of immunological challenge does not require that stability is maintained during infection. One way to experimentally determine how an acute challenge impacts the immune system in humans is to use vaccination. This enables the global assessment of the immune system prior to, during, and following a known immunological stimulus [14]. There is a general pattern that emerges from these systems vaccinology studies that demonstrates the elastic response of the human immune system to acute challenge. The early phase of the immune response (0-3 days post vaccination) is characterised by the expansion of circulating antigen-presenting innate immune cells, which activate T cells. The resultant expansion of T cells and the production of antibody-secreting plasma cells peak at 6-10 days post-vaccination, and are followed by a return to the pre-vaccination baseline [12, 13, 16-20]. The long-term functional memory T and B cells that persist [21, 22], are not present at a sufficient numbers to alter the cellular composition of the circulating immune system [12, 13, 16, 23-25]. In principle, an individual's immune profile could elastically return to the original stable state following this immunological challenge (**Figure 1c, Online Video 3**), or the transient changes caused during infection could cause it to settle into a new stable immunological state (**Figure 1d, Online Video 4**). The data for both gastrointestinal infection (traveller's diarrhoea) and influenza vaccination in people suggest that the 'elastic rebound' model is appropriate [13]. In other words, individuals will generally return to an immune phenotype similar to their original state after an acute immune stimulus, although chronic infections may be an exception (see dedicated section below). The presence of these two characteristics, longitudinal stability and elastic rebound after challenge, allows the further dissection of which factors shape diversity.

Intrinsic Factors Influencing the Human Immune System

Age

With advancing age the function of the immune system declines, rendering older persons more susceptible to infection and less able to generate protective immunity after vaccination [26]. Population immunology studies that have included age as a variable have found that aging impacts on ~20% of immunological traits, and overall

contributes ~5% of total immune variation [10, 11, 13, 27]. Analysis of the specific traits impacted demonstrates that the ageing human immune system is largely characterized by a decrease in the frequency of precursor cells, and an increase in the number of T cells with an activated or memory phenotype [10, 11, 13, 27, 28]. The changing immune landscape over time is likely driven by a number of biological mechanisms. As the immune system is constantly interacting with both pathogens and commensals, it is thought that these interactions play a key role in shaping the human immune system, such as by increasing the number of memory cells with age [29]. However, work with germ-free mice demonstrates that T cells that have not 'seen' their cognate antigen can acquire a memory phenotype via homeostatic expansion [30, 31]. Memory phenotype cells are found in human cord blood suggestive of homeostatic expansion [32], but the assumed sterility of the *in utero* environment is questionable [33]. Ageing is characterized by an increase in proinflammatory cytokines, which alter the environment of the immune system. This in turn may alter the phenotype and function of immune cells in an antigen-independent way [34]. Because humans maintain their naïve T cell pool for decades by peripheral T cell division alone [35], and as the long-term impact of a single immune challenge is minute in the immune landscapes of healthy people [12, 13], it is plausible that the age-dependent skewing of the immune system from a naïve to memory phenotype may occur, in part, without recognition of cognate antigen or as a result of cross-reactivity [36, 37].

Despite the clear role for non-genetic factors shaping the ageing immune system, cellular phenotypes associated with ageing, for example the decline in thymic output and thus numbers of recent thymic emigrants, have high heritability [10, 11, 13, 38]. This suggests that the interplay of genetic and non-genetic factors is important for the development of ageing phenotypes. While the role for selection of genes that impact ageing phenotypes is an area of intense debate [39], human longevity studies show a clear role of genetics in impacting how well we age [40]. Specific studies on how genetics shape the human immune system have yet to be realized. Nevertheless, single nucleotide polymorphisms associated with longevity are also linked with functional changes in the immune system [40-42], suggesting a link between immunity, inflammation and health in older age.

Sex

It is clear that there is sexual dimorphism in immune-related pathology. In general, autoimmune diseases are more common in women than men [43], while men are more susceptible to infections caused by viruses, bacteria, parasites and fungi [44]. There is also evidence that women produce higher antibody titers in response to vaccination than men, suggesting sex can impact immune function [44]. Despite clear differences in functional outcome, the differences in the cellular composition of the immune system between men and women are few; women tend to have a higher $CD4^+$ T cell count, and lower natural killer (NK) cell counts than men, but otherwise no consistent differences are observed [13, 27, 45], suggesting sex has a larger influence on disease outcome than immune composition. This is supported by large scale phenotyping of clinical and immunological parameters in parallel, that indicates that sex has the largest effect on clinical phenotypes [27]. Interestingly, the functional and cellular differences between the sexes are more pronounced in men with high testosterone [46], and disappear after menopause [44, 45], suggesting that the sex hormones are responsible for these changes, although precisely how this is mediated has yet to be elucidated.

Genetic Control Over Human Immune Variation

A likely driver for individual-to-individual variation in the settings of the immune system is genetic variation. Indeed, major studies into the genetic basis of variation in the cellular and molecular composition of the human immune system have found that genetic variation (e.g. common and rare variants, variations in copy number) accounts for 20-40% [10, 11, 47, 48] of total immunological variation (**Figure 2**). These studies have used either a twin or familial structure to estimate heritability. The twin studies use the classical ACE model (see **Glossary**), while Orrù *et al.* use a kinship matrix based on genotype to define relatedness [11]. Heritability estimates should therefore be considered with a broad definition of genetic variation, including (but not limited to) common, copy number and rare variants; heritable epigenetic modification and genotype-genotype interactions (for example, interactions between Killer immunoglobulin-like receptor gene variants and HLA variants determine the severity of CMV infection [49].

The range of heritability estimates, from 20 to 40% of total immunological variance, is likely to reflect both biological differences in the populations being measured and methodological differences in sampling and the choice of which immunological

parameters to include in the study of overall immune diversity. In this regard, it is notable that the highest estimates came from analysis of the Sardinian population (which is a genetic isolate). The lowest level of heritability was reported by Ye *et al.* [47]. There were several features unique to this study: (i) transcriptome-level analysis; (ii) tightly controlled sampling protocol (with only sampling between the hours of 07:30-08:30, from fasted participants); (iii) cells were examined both *ex vivo* and after stimulation and (iv) heritability was estimated from principal components based on ancestry. It is tempting, when comparing the lower heritability in Ye *et al.* to the other studies, to speculate that the transcriptome might well have a larger total variance than the cellular composition of the immune system. For instance, it seems plausible that the absolute expression of *CD4* mRNA may vary more markedly than the frequency of $CD4^+$ T cells. In support of this proposition, an older study using only basic immune populations found a much higher heritability estimate of ~70% [50]. This would indicate a hierarchy of genetic influence, with the strongest effects being felt at the leukocyte population level, moderate effects at the level of subsets and activation potential, and the weakest effects at the level of transcription. By inference, environmental effects are likely to be strongest at the molecular level and would have the weakest potential to influence major leukocyte populations. An exception may be cis-acting regulatory polymorphisms, with some of the highest heritability found for single protein expression markers and their corresponding gene (for example CD39 expression with *ENTPD1*, the CD39 gene, variants [38]). While it is debatable as to which level of immune variation is the most relevant, given that the basic unit of immunity is the cell it seems reasonable to us to focus on the variation present at the level of cellular subset and function, rather than lower-order (transcriptome, single gene expression) or higher-order (basic leukocytes) variation.

Beyond gross heritability analysis, several studies have explored the relationship between specific genetic variants and individual immune parameters. To date, only associations with common single nucleotide polymorphisms (SNP) have been explored [11, 38, 47]. The relatively low number of individuals assessed in this manner (compared to disease-based genome-wide association studies), and the multiple classes of genetic variants missing from these studies (**Box 3**), preclude final conclusions being made – there is more to be found from genetic analyses. However, as may be expected, many of the SNPs identified that are associated with changes in immune cell types are in or near genes with known functions in the immune system,

and in particular within genes that have been previously associated with autoimmunity, inflammatory diseases and susceptibility to infection [11, 38]. These findings strongly support the supposition that diversity in the immune system contributes to the relative risk of the individual to develop immunological disease.

Environmental Influences on Human Immune Variation

The estimate of the contribution heritable (20-40%) and intrinsic factors (~5%) make to the observed immunological variation suggests a major role for environmental factors. Unlike genetic and intrinsic factors, which are readily identifiable and measureable, dissecting out the individual environmental factors that may play a role is confounded by the limitless list of putative environmental factors that could be considered, the difficulty in collecting data for any of these factors, and the unknown timeframe over which environmental factors may be acting. Nevertheless, there are indications of the potency and nature of environmental contributors to immunological variation. We recently assessed the immunological variation that is present between opposite sex couples living with a child and found that the degree of variation was 50% lower than is observed in the general public [13], indicating that a convergence in immune status occurs during cohabitation (**Figure 3** and **online video 5**). Even with the proviso that couples are not randomly assorting, this data indicates that environmental factors are potent, that they can modify the adult immune system, and that they can be observed at the level of the household. The cohabitation effect is likely to be driven through the accumulated impact of multiple smaller factors, as the reduction of diversity evident within each couple was unique [13] (while convergence driven by a single environmental factor would be expected to show a consistent effect across multiple couples).

There are multiple promising candidates for the household environmental factors capable of shaping the variation present in the human immune system. One of the strongest is likely to be chronic viral infections, with discordance in cytomegalovirus infection a major driver of immune diversity between monozygotic twins [10] (see detailed section below). Other factors that are shared within household members and are known to alter the immune state, and which could therefore drive household-level immunological convergence, include smoking status [51], well-being [52, 53], pet ownership [54], physical activity [55], obesity [13], diet [56] and environmental pollutants [57, 58]. Each of these factors is also likely to influence the composition of

that individual's microbiota. The indirect microbiota convergence between individuals that share an environment (and thus the same microbiota modifiers) is also accompanied by direct transmission of microbiota between individuals in close proximity, enhancing microbiota convergence [59-62]. With the growing evidence that the microbiota composition, especially within the gut, influences the immune system [63-67], it is plausible that microbiota changes act as an integrator of multiple environmental factors, in turn shaping diversity within the human immune system. Seasonal change is another environmental factor that can influence immune variation [68], however it is unlikely to be captured in most study designs, as it would not induce variation between individuals living in the same region at a given time-point.

Bacteria and viruses are both able to establish chronic infections. For example *Mycobacterium tuberculosis* and cytomegalovirus (CMV) are able to persist long-term in humans. Such infections can be established when the pathogen is able to evade sterilizing immunity, followed by formation of an equilibrium between the pathogen and host in which the inflammatory response is reduced to a level that can control the pathogen without causing excess damage to the host [69, 70]. The balance between host and chronic infectious agents is a complex one; ultimately the pathogen requires a live host for its continued survival so must exist in a balance of not triggering overt immunity and clearance, and not overcoming the host with disease. This constant interaction with the host immune system has an indelible impact on the immune landscape.

There are a number of chronic viral infections that rarely cause disease in healthy hosts with symptoms only becoming apparent in immunocompromised individuals [69]. Despite the lack of clinical disease, this long-term dynamic interaction between host and virus has significant impact on altering the cellular composition of the immune system. The best characterised of these persistent infections are CMV and Epstein-Barr virus (EBV). CMV influences the immune system directly, by increasing the frequency of CMV-specific $CD4^+$ and $CD8^+$ T cells to around 5% of the total T cell pool in people under 55 years old [71], the frequency of CMV specific cells increases further with age suggesting a dynamic host-pathogen interaction that continues to shape the immune system [29]. This effect is likely due to the combined impact of thymic involution with the continued stimulation of CMV-specific T cells clones throughout life [72]. The effects of CMV are not limited to antigen-specific T cells; global analysis of the immune systems either unrelated individuals or monozygotic

twins that are discordant for CMV demonstrate that presence of this virus alters the level of certain serum cytokines, such as IL-6, and frequency of many cell types including γδ T cells, granulocytes and memory $CD8^+$ T cells [10, 27]. It is clear that CMV has significant effects on the composition of the immune system, and intriguing that it seems to be the most extreme example of a persistent infection impacting the host in this way. A caveat for the role of CMV in these studies, is that their concordance for other chronic infections was not assessed [10]. However, individuals infected with herpes simplex virus do not exhibit the type of changes in their circulating T cell compartment that are driven by CMV [73]. Persistent EBV infections that have not caused acute disease (infectious mononucleosis) also result in the accumulation of virus-specific $CD4^+$ and $CD8^+$ T cells, but not as high a frequency as CMV specific T cells [74, 75].

Concluding Remarks

The advent of large-scale high-depth immune profiling ("population immunology") has allowed a first insight into the scope and nature of immune diversity within the human population. In the case of systems vaccinology, this variation has a clear potential impact on health, with altered efficacy of vaccination against infectious diseases [12, 18]. However, variation in the human immune system is likely to not only influence responses to infections, but also to impact on susceptibility to autoimmunity, allergy, cancer and the inflammatory diseases of ageing, such as diabetes, cardiovascular disease and neurodegeneration. To a certain degree this variation, and the resulting disease susceptibility, is 'locked in', owing to the influence of genetic and intrinsic factors. However, a surprisingly high influence of environmental control has also been observed, such as is revealed by the effect of cohabitation [13]. New studies need to be performed to identify the distinct interactions between environment and immune variation, however for parameters such as microbiome [64], diet [56], air pollution [57] and even anxiety [53], it is likely that much of the influence they have over disease susceptibility will lie in their capacity to modulate the immune system. Dissections of the particular environment-immune interactions that constitute disease risk have the potential to allow targeted modification of the immune system by lifestyle and environmental modulation (see Outstanding Questions). Regulation of environmental influences over the immune system through approaches such as dietary modification, restricting exposure to certain pollutants and monitoring microbiota changes would combine aspects of personalized medicine with

preventative medicine, and could have a profound impact on the way immunological health is managed.

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Figure legends

Box 1. Limitations in SNP Genotyping Platforms

The SNP genotyping platforms typically used for genome-wide association studies place an emphasis on common single nucleotide (>5% allelic frequency), which accounts for ±90% of genetic variation in the human population [76]. This excludes certain classes of genetic variation, notably copy number variation and rare variants, which, while less common, may exert a disproportionate degree of influence over the immune system. SNP genotyping platforms can also struggle with immunological loci, owing to the enrichment for gene duplication events [77, 78]. For example, the rs1050501 SNP in *FCG2RB*, confers susceptibility to systemic lupus erythematosus and protection from malaria [79]. The pseudogene *FCGR2C* has an identical nucleotide sequence in this exon, but is homozygous for the ancestral allele. Genome-wide SNP platforms will discard rs1050501 at the quality control stages, as it will not follow Hardy-Weinberg equilibrium (that allele frequencies will remain constant across generations in the absence of selection pressure) due to contaminating signal from *FCGR2C*. These effects place a technical burden on estimating the contribution of such loci, which may cause an under-estimation of the genetic contribution of immunological loci.

Figure 1. Opposing Models for the Properties of Human Immune Variation. Variation in the immune system can be represented as different points that can be occupied on an 'immunological landscape'. **A.** The stochastic model is based on a fluctuating immune system, where apparent diversity is observed due to taking single snap-shots of an immune system in flux. **B.** The stability model is based on individual-to-individual variation reflecting a consistent longitudinal difference, with only minor changes occurring within healthy individuals monitored over time. The stability model raises additional possibilities of elastic or inelastic stability. **C.** The elastic stability model is based on individuals maintaining distinct immune variation,

consistently across time. While immunological disturbances (such as infection) can transiently alter the state, following the end of the disturbance, individuals 'rebound' to the original state. **D.** The inelastic stability model also has maintenance of distinct immune variation over time, however immunological challenges can disrupt the default state, resulting in a different stable state being achieved. The white flashes in C and D represent a immunological challenge, such as vaccination, or infection.

Figure 2. Heritability of Immune Traits Across Different Populations and Study Designs. Published heritability estimates for individual immune traits. For each study, the study design, numbers of individuals and measurement strategy (blue, twin study; orange, family study) is displayed. For Ye *et al.*, heritability estimates for each individual trait were unavailable; the 'global' reported estimate is shown; this study used a population study design. Boxes depict interquartile range (IQR) and median. The whiskers extend up to the furthest datapoint or 1.5 x IQR, in which case outliers are plotted with dots. Abbreviations used: MZ monozygotic; DZ dizygotic.

Figure 3. Interaction of Genetics, Age and Environment on Immune Variation. Immune variation can be modeled as an 'immunological landscape', with individuals occupying different points on the landscape. A synthesis of current data would place the role of genetics as influencing the initial starting point on the immune landscape. This initial variation is molded by age, with predictable changes occurring over time, driving the same changes in different individuals. Local environment strongly modifies other variables, leading to factors such as cohabitation (pink) resulting in convergence between different individuals to a closer point on the immunological landscape.

Online Video 1. The Stochastic Model of Human Immune Variation. Variation in the immune system can be represented as different points that can be occupied on an "immunological landscape". The stochastic model is based on a fluctuating immune system, where apparent diversity is observed due to taking single 'snap-shots' of an immune system in flux. Under this model, the relative diversity between individuals will change dramatically over time, as each individual constantly moves through the immunological landscape.

Online Video 2. The Stability Model of Human Immune Variation. Variation in the immune system can be represented as different points that can be occupied on an "immunological landscape". The stability model is based on individual-to-individual variation reflecting a consistent longitudinal difference, with only minor changes occurring within healthy individuals monitored over time. In this model, the immune system of individuals can vary with time, but this variation is largely contained to a limited region on the immunological landscape (visualized through a depression in the landscape). The current data obtained from population immunology studies supports this model over the one presented in Video 1.

Online Video 3. The Elastic Stability Model of Human Immune Variation. Variation in the immune system can be represented as different points that can be occupied on an "immunological landscape". The stability model is based on individual-to-individual variation reflecting a consistent longitudinal difference, with only minor changes occurring within healthy individuals monitored over time. In this model, the immune system of individuals can vary with time, but this variation is largely contained to a limited region on the immunological landscape during the homeostatic context (visualized through a depression in the landscape). Despite this stability during homeostasis, the immune system can radically change during infection (visualized through the individuals turning black). Under the elastic stability model, these immunological disturbances are only transient, and following the end of the disturbance, individuals "rebound" to the original homeostatic state. Current data seems to support this model in most cases.

Online Video 4. The Inelastic Stability Model of Human Immune Variation. Variation in the immune system can be represented as different points that can be occupied on an "immunological landscape". The stability model is based on individual-to-individual variation reflecting a consistent longitudinal difference, with only minor changes occurring within healthy individuals monitored over time. In this model, the immune system of individuals can vary with time, but this variation is largely contained to a limited region on the immunological landscape during the homeostatic context (visualized through a depression in the landscape). Despite this stability during homeostasis, the immune system can radically change during infection (visualized through the individuals turning black). Under the inelastic stability model, these immunological disturbances create a lasting imprint on the individual's

immune variation, with a new homeostatic state being established at the resolution of infection. Infections that can establish chronicity are a good example of this model.

Online Video 5. Interaction of Genetics, Age and Environment on Immune Variation. Immune variation can be modeled as an “immunological landscape”, with individuals occupying different points on the landscape. A synthesis of current data would place the role of genetics as influencing the initial homeostatic stability region on the immune landscape. Individuals show minor variation within their unique stability region over the short-term, however over the long-term the region of stability is molded by age, with predictable changes occurring over time, driving the same changes in different individuals (visualized by the downwards movement of the depression). In addition to the effect of age, local environment strongly modifies other variables, with factors such as cohabitation (with the pink flash indicating the initiation of cohabitation between the visualized individuals) resulting in convergence between the individuals to a closer point on the immunological landscape.

Glossary

Population immunology : The study of human immunology where the primary focus is to under the diversity of immune states and responses across a large population. Population immunology studies need to combine a high depth (e.g., detailed analysis of leukocyte population subsets or function) with large population size (typically between 100 and 1000 individuals) in order to identify the degree of immune variation and potential drivers.

ACE model: In the ACE model, total phenotypic variance is considered to be the sum of the variances attributed to genetic (A), common environmental (C) and unique environmental (E) factors. Twins share C and E, with monozygotic twins fully sharing A and dizygotic twins sharing 50%. Thus the assessment of multiple sets of monozygotic and dizygotic twins allows an estimation of the proportion of total phenotypic variance that is driven by genetic variation.