Human Immunology through the Lens of Evolutionary Genetics

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Pathogen-imposed selection pressures have been paramount during human evolution. Detecting such selection signatures in ancient and modern human genomes can thus help us to identify genes of temporal and spatial immunological relevance. Admixture with ancient hominins and between human populations has been a source of genetic diversity open to selection by infections. Furthermore, cultural transitions, such as the advent of agriculture, have exposed humans to new microbial threats, with impacts on host defense mechanisms. The integration of population genetics and systems immunology holds great promise for the increased understanding of the factors driving immune response variation between individuals and populations.

Introduction

Humans and microbes have a long-standing, double-edged relationship. They can complement each other, as for the mutualist gut microbiota, but in other situations, microorganisms can be pathogenic, causing disease in the human host. Like famines and wars, which have continually inflicted a massive mortality burden on humans, the threat of pathogenic infections has had an overwhelming effect (Cairns, 1997). Pathogens have accompanied humans since their emergence in Africa ~200,000-300,000 years ago (ya), through subsequent dispersals across continents over the last ~60,000-100,000 years, and major cultural transitions, such as the emergence of agriculture and sedentarism ~10,000 ya, until more recent migratory events, such as the European colonization of the Americas and contemporary globalization (see Karlsson et al., 2014, and references therein). Ancient diseases, such as malaria and tuberculosis, the causal agents of which emerged ~100,000-70,000 ya, and more recent diseases, such as Black Death and Spanish flu, have wiped out hundreds of millions of people during human history (Comas et al., 2013; Fumagalli and Sironi, 2014; Morens et al., 2008). Mortality rates from infection remained high until the late 19th and early 20th century, when hygiene improved and vaccines and antibiotics began to be developed, and they remain high in many developing countries.

Infectious diseases have been, and still are, a major cause of human mortality, and thus, they represent a strong selection pressure. For example, at the end of the 19th century, only 35% of Europeans reached the age of 40 years, highlighting the high infectious burden and illustrating what must have been the rule during most of our history as a species (Cairns, 1997; Casanova and Abel, 2005). John B. S. Haldane and Anthony Allison were the first to establish a formal link between infectious diseases and natural selection, with the suggestion that red blood-cell disorders, such as thalassemia and sickle-cell disease, can provide protection against malaria (Allison, 1954; Haldane, 1949). Strong evidence concerning the genetic determinants of infectious, inflammatory and autoimmune disorders has since accumulated (Abel et al., 2014; Parkes et al., 2013). The genome-wide approaches

developed over the last decade, in particular, have revealed that the observed inter-individual heterogeneity in infection outcome is due, in many cases, to differences in the genetic make-up of the human host, resulting in rare, Mendelian diseases or altering the individual susceptibility to complex immune-related phenotypes (Casanova and Abel, 2018).

Population genetic studies have shown that genes involved in immune function present strong selection signatures, have helped to delineate genes and pathways of major importance in host defense, and have provided support for the notion that microbes have had an overwhelming impact on human evolution (Barreiro and Quintana-Murci, 2010; Fumagalli and Sironi, 2014; Karlsson et al., 2013; Quintana-Murci and Clark, 2013). This review does not attempt to provide a comprehensive view of the effects of different types of selection on genome diversity or the statistical methods available to detect them, which have already been dealt with in outstanding reviews (Fan et al., 2016; Vitti et al., 2013). Likewise, much of the excellent work on the population genetics of immune system genes in humans will not be reviewed here, as this work has already been extensively reviewed elsewhere (Barreiro and Quintana-Murci, 2010; Fumagalli and Sironi, 2014; Karlsson et al., 2014; Quintana-Murci and Clark, 2013).

Instead, I will first provide an overview of the way in which the detection of different selection regimes, from maintenance of the *status quo* to the benefits of diversity, can provide us with information about the immunological relevance of host genes in natural settings, stressing the value of studies of ancient DNA time transects for the direct detection of natural selection. I will also provide an extensive review of how admixture at different time scales — from archaic admixture with ancient hominins such as Neanderthals to more recent historical gene flow between human populations — can provide an additional source of advantageous immune variation. In addition, I will describe how changes in subsistence patterns, such as the transition from nomadic hunting and gathering to a sedentary farming-based lifestyle, may have modified the way humans interact with pathogens. Finally, I will highlight the ways in which the integration of population genetics and systems immunology can help us to tackle questions of major fundamental and public health importance, such as

the delineation of the evolutionary determinants of human immune responsiveness, and the various sources of immune response variation between both individuals and populations.

Population Genetics of Human Immunity — from Essentiality to Redundancy

A dissection of the molecular signatures left by natural selection in genomic regions involved in immune responses, or the absence of such signatures (i.e., neutrality), can be used to assess the biological relevance of the corresponding genes *in natura,* determining whether they are essential, redundant or adaptable (**Figure 1**). Two main forms of selection occur, according to whether the alleles concerned are deleterious or advantageous. Purifying selection, or negative selection, involves the removal of deleterious alleles from the population. Its pace depends on how deleterious the alleles are (i.e., the selective coefficient *s*) and on effective population size (i.e. N_e). This selection regime is the most widespread throughout the genome. Conversely, adaptation can occur through positive selection, which increases the frequency of an advantageous allele according to various evolutionary models such as the classic sweep (strong selection on a new mutation), standing variation (selection on a pre-existing mutation), or polygenic adaptation (selection acting simultaneously on multiple loci) (**Figure 2A**), or through other forms, such as balancing selection or adaptation through admixture (**Figure 2B,C**), which we will discuss in detail later in this review.

The genes evolving under strong purifying selection are those for which functional variation cannot be tolerated. These genes thus encode products with essential, non-redundant functions, and their mutations may underlie severe disorders. Innate immunity genes are generally constrained by selection, particularly those associated with autosomal dominant primary immunodeficiencies, such as *STAT1* and *TRAF3* (Deschamps et al., 2016). Classical examples of highly constrained immune genes include the genes encoding endosomal Toll-like receptors (TLRs) (Barreiro et al., 2009), most NALP members of the NOD-like receptor (NLR) family (Vasseur et al., 2012), IFN-γ (Manry et al., 2011), and many others (Quintana-Murci and Clark, 2013). Mutations of some of these genes or affecting the

pathways they trigger (e.g., TLR3-TRIF, TIR-MYD88, IFN-γ, NALP3) have been associated with life-threatening phenotypes, such as HSV-1 encephalitis, pyogenic bacterial infections, Mendelian susceptibility to mycobacterial disease, and severe inflammatory diseases (for an extensive review, see Casanova and Abel, 2013).

Strong or complete redundancy can also be deduced from population genetics data. Redundant genes often display profiles of genetic diversity that are consistent with relaxed selective constraints. This is the case for the three members of the RIG-I-like receptor (RLR) family, suggesting some degree of redundancy in immunity to viruses (Barreiro et al., 2009; Vasseur et al., 2012). High redundancy is illustrated by cases of apparent "knockouts" in humans, as reported for *IFNA10*, *IFNE*, *MBL2*, and *TLR5*, loss-of-function variants of which can reach high frequencies in the healthy general population (Quintana-Murci and Clark, 2013). In rare cases, loss-of-function may not only be tolerated, but may actually prove beneficial, with disruptive variants increasing to high population frequencies through positive selection. This situation has been reported for *CASP12*, *DARC* or *FUT2*, possibly because they provide protection against sepsis, *vivax* malaria and noroviruses, respectively (Casanova and Abel, 2018; Quintana-Murci and Barreiro, 2010).

Host Genetic Adaptation to Infectious Agents at Different Time Scales

The detection of different forms of adaptive evolution (**Figure 2**) makes it possible to identify genes and functions for which novelty or diversity has been beneficial to the host (Karlsson et al., 2014; Key et al., 2014; Quintana-Murci and Clark, 2013). In particular, there is growing evidence confirming that the diversity of immune genes is driven by human exposure to pathogens. For example, significant correlations have been detected between the genetic variability of *HLA* class I genes, blood group antigens, and interleukin genes and pathogen diversity worldwide (Fumagalli et al., 2009a; Fumagalli et al., 2009b; Prugnolle et al., 2005). Some of these correlations may be due to confounding with variables such as climate, diet or lifestyle, but the primary drivers of local adaptation have been shown to be pathogens, the

diversity of which is correlated with that of the inflammatory response and innate immunity genes (Fumagalli et al., 2011). It has recently been suggested that viruses have been the most pervasive agents driving mammalian evolution, with 30% of all amino-acid changes in the human proteome due to selection pressures imposed by viruses (Enard et al., 2016).

Adaptation to pathogens may in some cases cause collateral damage. There is increasing evidence to suggest that past selection for higher resistance to infection may result — following the decreasing incidence of infections — in present-day susceptibility to autoimmune, inflammatory, or allergic diseases (Sironi and Clerici, 2010), as predicted by the hygiene hypothesis (Strachan, 1989). Signatures of positive selection have been reported for genetic variants that are associated with increased risk of inflammatory bowel disease, type 1 diabetes, celiac disease, multiple sclerosis, and psoriasis among others (see Brinkworth and Barreiro, 2014, and references therein). However, because the genetic architecture of polygenic traits, such as immunity to infection, is likely to be dominated by the effects of long-term stabilizing selection (Simons et al., 2018) and pleiotropy is pervasive among immune genes (Brinkworth and Barreiro, 2014), establishing a direct link between past selection favoring a specific phenotype and present-day maladaptation is far from straight-forward.

Preserving Immune Diversity Over Long Time Periods

Balancing selection may take various forms and acts to preserve functional, beneficial genetic diversity. The mechanisms of balancing selection include overdominance (i.e., heterozygote advantage), frequency-dependent selection (i.e., rare allele advantage), and selection fluctuating over time and space (Key et al., 2014). There is increasing evidence for long-lived balancing selection, a selective regime allowing polymorphisms to survive over long time periods and during speciation (trans-species polymorphism) (**Figure 2B**). Textbook examples of balancing selection can again be found in the immunological literature, in the form of the extraordinarily high levels of genetic diversity reported for the vertebrate major histocompatibility complex (MHC, referred to as HLA in humans), genetic variation in which has been maintained and inherited from distant ancestors in primates, but also in other

mammals, birds and fish species (Klein et al., 2007; Lawlor et al., 1988). Likewise, the ABO histoblood group was recently shown to be a trans-species polymorphism, identical by descent in various primate species, including humans and gibbons in particular (Segurel et al., 2012). Other factors have helped to maintain high levels of diversity and trans-species polymorphism at the *HLA* and *ABO* loci, including sexual selection in the case of HLA (Ober et al., 1997), but the selection signals observed are probably pathogen-driven (Prugnolle et al., 2005).

Other immune genes have been shown to evolve through the action of balancing selection on functions relating to cell migration and β-defensins (Cagliani et al., 2008). That this selective regime is particularly pervasive in the context of immune function is supported by genome-wide studies, which have explored the extent of balancing selection long-lived and trans-species (Leffler et al., 2013; Teixeira et al., 2015), within humans (Andres et al., 2009; DeGiorgio et al., 2014; Siewert and Voight, 2017), as well as more complex models of balancing selection turning into positive selection (de Filippo et al., 2016). Several immune functions involving genes encoding membrane glycoproteins, such as *GYPE,* and innate immunity proteins, such as *IGFBP7,* present strong signals of trans-species polymorphism between humans and chimpanzees (Leffler et al., 2013). Within humans, genes subject to balancing selection include not only *HLA* but also *BTN1A1*, *NALP13*, *TRIM22* and *FUT2*, for example (Andres et al., 2009). Together, these studies show that even though balancing selection remains rare, it has shaped the diversity of gene functions relating to immune responses and host-pathogen interactions (Key et al., 2014).

Recent, Local Adaptation to Pathogen Pressures

Unlike balancing selection, which maintains a mutation at a particular frequency equilibrium, positive directional selection acts on newly occurring or previously neutral mutations, leading to an increase in the frequency of these mutations, potentially to fixation. Over the last 60,000 years or so, as modern human populations dispersed around the globe, they were confronted with diverse local environments, leading to population- or region-specific genetic

adaptation. Indeed, one of the classic signatures of local adaptation is an increase in population differentiation, a pattern that can be detected using F_{ST}-based methods, which measure the part of genetic variability that is explained by population divergence (i.e., heterozygosity within and between populations) (Fan et al., 2016; Vitti et al., 2013). Signals of local adaptation have been found in genes influencing phenotypes that are variable across populations, such as skin pigmentation, height, BMI, lactose tolerance, starchy food digestion, and immune response (Fan et al., 2016), but also in populations adapted to extreme physiological conditions, such as life in the Artic or at high altitude (Ilardo and Nielsen, 2018).

The list of immune genes and functions known to be subject to selection is continually increasing, with some signals supported by functional or epidemiological data. Classic cases of local adaptation include variants of genes associated with resistance to malaria in Africa and Asia (e.g., *DARC*, *G6PD, GYPA*, *GYPB*, *GYPE*) and *Trypanosoma* infection in Africa (*APOL1*), weaker inflammation/NF-κB signaling in Europe (*TLR10-TLR1-TLR6* cluster) and Africa (*TLR5*), antiviral responses in Europe and Asia (type-III IFNs), *Vibrio cholerae* infection in Bangladesh (NF-kB signaling pathway genes), and immunity to Lassa virus infection in Africa (*LARGE* and *IL21*) (see Brinkworth and Barreiro, 2014; Fumagalli and Sironi, 2014; Grossman et al., 2013; Karlsson et al., 2014; Quintana-Murci and Clark, 2013, and references therein). Other interesting cases of positive selection requiring further investigation include *MERTK* in Asians and *ZFPM2* in Africans, which have been associated with hepatitis C-induced liver fibrosis and tuberculosis, respectively (Deschamps et al., 2016).

Signals of local adaptation can also be very recent and geographically confined. This is the case for *CD36*, which encodes a pattern recognition receptor mediating the cytoadhesion of *Plasmodium falciparum*-parasitized erythrocytes. This gene presents robust signals of positive selection targeting a stop mutation restricted to West Africans (29% in the Yoruba from Nigeria), and the selection event has been dated back to 3,600 ya (Deschamps et al., 2016; Fry et al., 2009). The use of a new metrics developed to detect selection over the last

2,000 years has also led to the detection of very recent selection in the *HLA* region (Field et al., 2016), illustrating the action of selection in historical times.

Adaptation to pathogens can follow evolutionary trajectories other than classic trans-species balancing selection or recent positive selection. This is the case for the recent balancing selection regime acting on the β-globin gene (*HBB*) in Africa (**Figure 2B**). This is one of the most striking examples of natural selection maintaining a deleterious mutation at high frequency in the population, owing to the heterozygote advantage afforded by the β^S sickle (HbS) mutation in regions in which malaria is endemic (Allison, 1954). HbS/HbS homozygotes develop sickle-cell disease, an often-fatal form of anemia caused by red-cell deformities, and HbA/HbA homozygotes are susceptible to malaria. By contrast, heterozygotes are about an order of magnitude less susceptible than HbA/HbA homozygotes to life-threatening forms of malaria (Ackerman et al., 2005; Allison, 1954).

Insights from Ancient DNA — Measuring Selection in Time Transects

For many years, our understanding of human adaptation to environmental changes was limited to statistical inferences from patterns of genetic variation in contemporary populations. Over the last decade, the use of ancient DNA from modern humans has revolutionized the study of human prehistory, but it has also opened up new opportunities for measuring the action of natural selection directly (Skoglund and Mathieson, 2018). Such direct measurements are based on the detection of allele frequency changes in samples from populations before, during and after specific events requiring adaptation, such as the advent of agriculture. As in studies based on modern DNA, the clearest evidence of selection obtained with ancient DNA data concerns the development of lactose tolerance in Europe, together with fatty acid metabolism, indicating that the frequency of the lactase persistence allele has also increased very recently, during the last ~4,000 years (Mathieson et al., 2015; Skoglund and Mathieson, 2018). These ancient DNA studies confirm the high selective coefficient *s* of the lactase persistence allele estimated from modern DNA data (*s:* 0.025 to 0.069) (Peter et al., 2012; Tishkoff et al., 2007); strong selective coefficients are indeed

needed to detect allele frequency changes in the relatively short time evolutionary periods covered by ancient DNA analyses.

Ancient DNA data have also increased our understanding of the evolution of the immune system. Based on data from 230 Eurasians living between 8,500 and 2,300 ya, variation at the *TLR10-TLR1-TLR6* cluster has been shown to be a strong substrate for positive selection in Europe (Mathieson et al., 2015), possibly reflecting, although no evidence for this exists, adaptation to infectious agents causing diseases such as leprosy or tuberculosis (Mathieson et al., 2015). Likewise, strong selection signals have been detected at the *HLA* and *SLC22A4* loci, both of which are associated with immune phenotypes, consistent with the occurrence of pathogen-related adaptation during the Holocene period. This notion is supported by another study, based on a 7,000-year-old Mesolithic skeleton, which suggested that the adaptation of immune-related traits through changes in genes such as *TLR1*, *CD14*, *IFIH1*, *CASP12* and *NOS2A* occurred before changes in skin pigmentation, at least in Europe (Olalde et al., 2014). Such adaptation may even have begun before the Neolithic period, as suggested by *TLR10-TLR1-TLR6* and *HLA* data for Pleistocene North African genomes dating back to ~15,000 ya (van de Loosdrecht et al., 2018).

The arrival of Europeans in the Americas, and the subsequent decline of Native American populations, has been linked to the introduction of new pathogens into immunologically naïve indigenous populations (Thornton, 1997). One recent study explored a time transect of exomes from a continuous population from the Northwest Coast of North America, dating from before and after the first contact with Europeans (Lindo et al., 2016). Selection signals have been detected in immune genes in ancient Native American samples, particularly at *HLA-DQA1*, reflecting possible adaptation to pathogens present in the ancient environment. The selected alleles have a much lower frequency in the modern population, suggesting that the arrival of the Europeans triggered environmental changes rendering these genetic variants deleterious and subject to negative selection in native populations.

Ancient DNA data can also be used to investigate negative selection, providing information about the deleteriousness of allelic variants, as illustrated by a recent study of

mycobacterial infections (Boisson-Dupuis et al., 2018). A missense variant of *TYK2* (P1104A) has been shown to confer predisposition to tuberculosis, and the frequency of this variant in Europe has decreased from ~9% to 4% over the last 4,000 years, consistent with this variant being purged from the population by endemic tuberculosis. Collectively, these studies highlight the value of ancient DNA data for direct measurements of the action of selection over time, and for identifying and quantifying the contribution of selection to immune-related phenotypic variation.

Polygenic Signals of Adaptation

The examples from ancient and modern DNA data discussed above illustrate the adaptability of immune response genes, through the action of strong positive or balancing selection. However, these selection regimes remain relatively rare in humans (Hernandez et al., 2011; Key et al., 2014). A subtler mechanism of adaptation is *polygenic adaptation*, in which minor allele frequency changes at multiple loci contribute to the adaptive phenotype (Pritchard et al., 2010) (**Figure 2A**). Signals of this selective regime have been reported for various phenotypes, including height, skin pigmentation, adaptation to high altitude, birth weight, sexual maturation, and educational attainment (Berg and Coop, 2014; Field et al., 2016; Gouy et al., 2017; Racimo et al., 2018; Turchin et al., 2012). However, the polygenic adaptation signal for height, which was long thought to be strong, has been called into question by a recent study (Berg et al., 2018). Focusing on host defense, gene sets and immune response-related pathways associated with the *IL-6 signaling pathway*, *malaria*, *cytokine–cytokine receptor interaction*, and *pathogenic Escherichia coli infection* have been shown to display polygenic adaptation signals (Daub et al., 2013). The significance of most of these gene sets is not due to strong signals for a few genes but to small effects on multiple loci, providing strong support for the hypothesis that pathogen-driven selection has been common in the human genome (Barreiro and Quintana-Murci, 2010; Fumagalli et al., 2011) and that adaptation to pathogens is a primary example of polygenic selection in humans.

Acquiring Advantageous Immune Variation Through Admixture

Beneficial genetic variation can be acquired from other populations or species through admixture. Adaptive variants from donor populations can be introgressed into recipient populations or species (**Figure 2C**). This process, known as *adaptive introgression*, is considered to be a source of adaptive alleles by theoretical population geneticists, with empirical support being obtained in several plants and animals (Hedrick, 2013; Rieseberg, 2009). However, we have only recently begun to assess the extent of adaptive introgression in humans, either from archaic hominins or between modern human populations.

Admixture between Archaic and Modern Humans: Adaptive Introgression

The possibility of sequencing genomes from ancient hominins such as Neanderthals and Denisovans has generated unprecedented insight into admixture between modern humans and ancient hominins (Dannemann and Racimo, 2018; Vattathil and Akey, 2015). Three high-coverage genomes of archaic humans are currently available, corresponding to a Neanderthal and a Denisovan from the Altai Mountains (Meyer et al., 2012; Prufer et al., 2014), and a Neanderthal from Croatia (Prufer et al., 2017), and several low-coverage genomes have also been obtained for Neanderthals living 39,000 to 47,000 years ago (Hajdinjak et al., 2018). The ancestors of non-Africans admixed with these archaic hominins, resulting in ~2% Neanderthal ancestry in the genomes of Eurasians, <1% Denisovan ancestry in East and South East Asians, and up to 6% Denisovan ancestry in some Oceanian populations (Dannemann and Racimo, 2018). Interestingly, some specific regions of the genome of present-day populations can show up to 64% of archaic ancestry (Sankararaman et al., 2014).

It is becoming increasingly clear that, in most cases, archaic introgression was selected against, leading to a depletion of Neanderthal ancestry in conserved genomic regions (Petr et al., 2019; Sankararaman et al., 2014; Sankararaman et al., 2016). Conversely, high levels of archaic ancestry in modern populations indicate either a tolerance of archaic variants (i.e., neutrally evolving variants that did not affect fitness) or the selection of these variants (i.e.,

variants increasing the fitness of modern humans after admixture). Cases of archaic introgression that appear to be adaptive have been reported for genes relating to body morphology, metabolism, and responses to environmental conditions, such as temperature, altitude, sunlight, and pathogens (Gittelman et al., 2016; Racimo et al., 2017).

The first link between archaic introgression and immunity identified was that reported for the *HLA* region (Abi-Rached et al., 2011). Several HLA haplotypes, some of which encode ligands for natural killer cell receptors (KIRs), were acquired through admixture with Denisovans or Neanderthals. Since this early study, the number of immune genes found to present evidence of beneficial archaic introgression has continually increased (Dannemann et al., 2016; Deschamps et al., 2016; Enard and Petrov, 2018; Gittelman et al., 2016; Mendez et al., 2012, 2013; Nédelec et al., 2016; Sams et al., 2016; Sankararaman et al., 2014). Levels of Neanderthal ancestry are generally high for innate immunity genes, with the antiviral *OAS* cluster, the restriction factors *IFITM1-3* and the cytokines *IL17A* and *IL17F*, for example, displaying the highest levels of Neanderthal introgression at the genome-wide scale (Deschamps et al., 2016). Furthermore, the high levels of archaic ancestry of some of these genes may reflect adaptive introgression, as reported for *OAS1-3* (Gittelman et al., 2016; Mendez et al., 2013; Racimo et al., 2017; Sams et al., 2016; Sankararaman et al., 2014), *STAT2* (Mendez et al., 2012), the *TLR6-1-10* cluster (Dannemann et al., 2016; Deschamps et al., 2016; Gittelman et al., 2016), and *TNFAIP3* (Gittelman et al., 2016). Some of these archaic haplotypes may reach high frequencies in the population, as reported, for example, for *OAS1-3* in Europe (~36%), *TLR6-1-10* in East Asia (~39%) and *TNFAIP3* in Melanesians (~60%) (Gittelman et al., 2016; Racimo et al., 2017).

Some introgressed archaic haplotypes have been reported to affect molecular phenotypes, such as gene expression (Dannemann et al., 2017; McCoy et al., 2017). For example, introgressed Neanderthal variants have been shown to display allele-specific expression, particularly in the brain and testes, in which these variants are strongly downregulated, but nevertheless affect immune phenotypes, such as the expression of *TLR1* and *OAS1-3* (Dannemann et al., 2016; Dannemann et al., 2017; McCoy et al., 2017; Sams et

al., 2016). One very frequent Neandertal haplotype at the *OAS* cluster has been associated with lower levels of *OAS3* expression in response to infection (Sams et al., 2016). Likewise, Neanderthal alleles in present-day individuals of European ancestry are associated with variations in the transcription of genes expressed in macrophages and monocytes, particularly those involved in the response to viral challenges (Nédelec et al., 2016; Quach et al., 2016). Further support for a link between archaic introgression and the antiviral response was provided by a recent study showing that segments introgressed from Neanderthals are enriched in genes encoding proteins that interact with RNA viruses (Enard and Petrov, 2018). Additional evidence for an effect of archaic variants on ultimate phenotypes has come from various studies reporting overlaps between these variants and associations with various phenotypes, many but not all of which are immune-related, such as lupus erythematosus, other autoimmune disorders, and allergies (Dannemann et al., 2016; Dannemann and Kelso, 2017; Sankararaman et al., 2014; Simonti et al., 2016).

Admixture Between Modern Human Populations: Adaptive Admixture

Archaic introgression is increasingly being recognized as a source of adaptive variation, but the role of between-population admixture in human adaptation, a process known as *adaptive admixture*, remains largely unknown. An atlas of human admixture history, based on genome-wide data from 95 different populations, revealed that admixture events had occurred in most human populations in the last few thousand years (~4,000 years) (Hellenthal et al., 2014). Classic examples of admixture between expanding populations and local groups at different time scales include the impact of the Mongol Empire, the Arab slave trade, the Bantu expansions, and much more recently, European colonialism. However, we have only recently begun to consider whether such admixture events brought new advantageous variation.

The history of the African continent has been marked by admixture events at different time scales, and African genomes contain segments derived from multiple ancestries (Busby et al., 2016; Patin et al., 2017; Patin et al., 2014; Pickrell et al., 2012; Schlebusch et al., 2012;

Skoglund et al., 2017). Linguistic and archaeological records indicate that Bantu languages expanded, together agriculture, from western central Africa to southern Africa, about 4000 to 5000 ya (Phillipson, 2005). Population genetic studies showed that this spread was accompanied by the dispersal of people, who admixed, to various extents, with the local populations they encountered (Busby et al., 2016). Two studies revealed that gene flow and admixture within Africa have been accompanied by the exchange of adaptive variants (Busby et al., 2017; Patin et al., 2017). For example, during their dispersal through the rainforest, western Bantu-speaking groups encountered local populations of rainforest hunter-gatherers, with whom they admixed ~800 ya and from whom they acquired beneficial alleles in the *HLA* gene region (Patin et al., 2017) (**Figure 3**).

Other studies have provided additional support for the role of *HLA* as a key substrate for adaptive admixture (Busby et al., 2017). Some *HLA* haplotypes have been targeted by selection since admixture, as recently as the last few hundred years, as attested by the excess of African ancestry at the *HLA* locus in some populations from Mexico, Puerto Rico, Colombia and other admixed Hispanic/Latino groups (Rishishwar et al., 2015; Tang et al., 2007; Zhou et al., 2016). These results support the notion that the *HLA* region is a selection hotspot, consistent with the differential retention of *HLA* haplotypes in admixed populations from America, possibly due to the pathogenic environment of the New Word. In this context, two recent studies reported an association between African ancestry and a protective effect against severe dengue fever in admixed populations from Cuba and Colombia (Chacon-Duque et al., 2014; Sierra et al., 2017).

The case of the Fulani from The Gambia, a population resulting from an admixture event ~1,800 ya involving individuals of Eurasian and West African origin, is also worth discussing. Since the admixture event, the Fulani have retained high levels of African ancestry for the *DARC* gene, the null allele of which confers resistance to *Plasmodium vivax* malaria, and high levels of Eurasian ancestry at the *LCT* locus, which contains a lactase persistence allele facilitating the digestion of milk in adults (Busby et al., 2017). The highly adaptive nature of the Duffy-null allele and its rapid spread across populations via admixture is supported by

other studies reporting high African ancestry in admixed populations from Pakistan and Madagascar, where *vivax* malaria is endemic (Hodgson et al., 2014; Laso-Jadart et al., 2017; Pierron et al., 2018). These studies provide proof-of-concept that host adaptation to pathogens can be accelerated by gene flow, but they are generally restricted to a few cases in populations of African descent. The systematic detection of genomic signatures of adaptive admixture in human populations worldwide is now warranted, to determine the role of this process in the evolution of immune-related traits and inform disease risk.

Cultural Transitions and Host Immune Adaptation

The transition from food collection (hunting and gathering) to food production (farming and herding) during the Neolithic period was probably the most important innovation in human history. The shift to agriculture led humans to adopt sedentary lifestyles, in turn resulting in increases in population densities, and changes to the chemical, nutritional and pathogenic environment (Diamond and Bellwood, 2003). It has been suggested that the development of agriculture facilitated the spread of certain infectious agents, and that exposure to new zoonoses occurred following the domestication of animals (Wolfe et al., 2007). Evidence suggesting that this transition imposed new pathogenic pressures is provided by population genetic data for innate immunity genes, with most of the selection events occurring ~6,000–13,000 ya (Deschamps et al., 2016).

Studies of selection intensity in the genomes of populations with different subsistence patterns should provide us with additional, more direct information about the biological functions involved in adaptive processes relating to lifestyle changes and, importantly, inform health-related issues in populations exposed to different ecologies. In this context, engaging different human populations, in particular neglected indigenous groups, in genomics research is important not only to strengthen their representation in studies of human diversity but, notably, to increase knowledge on the links between genetic diversity and disease risk, a field that has been overly dominated by studies in individuals of European ancestry.

Africa, in particular, harbors the largest group of active hunter-gatherers anywhere in the world — the rainforest hunter-gatherers (historically referred to as “pygmies”) — and Bantu-speaking agrarian communities (Hewlett, 2014). Not only do these two groups have different subsistence patterns, but they also differ in their ecologies and exposure to environmental pressures and diseases. The last decade has revealed much about the demographic history of these populations in terms of past population splits, temporal changes in population sizes and admixture (reviewed in Patin and Quintana-Murci, 2018). The ancestors of rainforest hunter-gatherers and farmers diverged > 60,000 ya (Hsieh et al., 2016; Lopez et al., 2018; Patin et al., 2009; Verdu et al., 2009), so these two populations probably have a long history of adaptation to different ecological settings. In rainforest hunter-gatherers, selection signals have been reported for height, a phenotype that seems to be polygenic and evolving convergently (Bergey et al., 2018; Perry et al., 2014), and for functions relating to cell signaling, neural development and immune functions (e.g. antigen binding and pattern recognition receptor activity) (Hsieh et al., 2016; Lachance et al., 2012). In farmers, most, but not all of the candidate genes for adaptation are related to malaria resistance (*HBB*, *DARC*, *G6PD*, *CR1* and *CD36*), particularly in western and central Africa (Gurdasani et al., 2015; Patin et al., 2017), highlighting the link between deforestation and increased exposure to malaria.

A recent study revisited the history of the balancing selection acting on the *HBB* gene in Africa, and showed that the sickle-cell mutation first appeared in the ancestors of present-day farmers more than 20,000 ya (Laval et al., 2019). It was then acquired by rainforest hunter-gatherers through adaptive admixture over the last ~6,000 years. These findings suggest that the ancestors of current farming populations may have been exposed to malaria earlier than was previously appreciated (Shriner and Rotimi, 2018).

Another study explored adaptation to infectious diseases in two African San groups of hunter-gatherers: the ǂKhomani, who have had extensive contact with incoming farmers and herders, and the more isolated Ju|’hoansi (Owers et al., 2017). Interestingly, immune genes showed strong signals of selection in the ǂKhomani but not in the Ju|’hoansi, suggesting that

the adaptation of immune functions can be rapidly triggered by contact with external groups importing new pathogens (**Figure 3**).

These studies suggest that differences in lifestyle are associated with different histories of host adaptation to pathogens, but little is known about the underlying phenotypes potentially accounting for the molecular signals of natural selection observed. A recent study combined population genetics and functional immunology to address this question, focusing on the rainforest Batwa hunter-gatherers and the neighboring Bakiga famers from Uganda (Harrison et al., 2018). Using an *ex vivo* cellular approach, the authors showed that the largest differences in immune response between these two groups were related to viral rather than bacterial stimuli, and that the signals of positive selection accounting for these population differences were disproportionately observed among hunter-gatherers. Although a number of possible confounders must be considered, these results suggest that pathogen exposure is driven principally by ecological factors rather than by mode of subsistence (i.e., the advent of agriculture as proposed by (Diamond and Bellwood, 2003)).

That lifestyle and environmental factors can also have a major impact on immune gene expression — stronger than genetic variation — has been reported in populations with contrasting lifestyles from North Africa, where a substantial fraction of transcriptional variation is dictated by the urban versus rural exposure of these populations (Idaghdour et al., 2010) (**BOX 1**). This observation is supported by recent findings in a founder population from Québec, where environmental factors, such as air pollution, have been found not only to have an important impact on gene expression but also to modulate the penetrance of genetic variants (Fave et al., 2018). These studies collectively indicate that population differences in lifestyles and ecologies, including those that are pathogen-related, can impact immune responses and modulate the effects of genetic variation and disease risk.

Individual- and Population-Level Variation of Immune Phenotypes

Population genetics studies have identified key evolutionary determinants of the human immune system, but the links between genetic diversity, whether neutral or targeted by

natural selection, and immune phenotypes remain largely unexplored. Genome-wide association studies (GWAS) have provided new insight into the genetic determinants of immune response variation, including disease-related variation (Abel et al., 2014; Casanova and Abel, 2018; Parkes et al., 2013). Most such studies have also shown trait-associated hits to be located in regulatory regions (Nicolae et al., 2010; Pickrell, 2014). In this context, the biomedical value of mapping the genetic determinants of gene expression variation (i.e., expression quantitative trait loci, eQTLs) has become apparent in recent years, as these regulatory variants have proved informative for establishing links between genetic variation, intermediate phenotypes, such as gene expression, and ultimate phenotypes, such as immunity to infection or disease-related traits (Fairfax and Knight, 2014; Schmiedel et al., 2018).

Various studies have mapped eQTLs in contexts relevant to immunity, with the measurement, in particular, of expression levels in the presence of immune (e.g., TLR ligands, IFNβ, IFNγ) or infectious (e.g., influenza A virus, *Mycobacterium tuberculosis*, *Listeria monocytogenes*) stimuli (Alasoo et al., 2018; Barreiro et al., 2012; Caliskan et al., 2015; Fairfax et al., 2014; Kim-Hellmuth et al., 2017; Lee et al., 2014; Nédelec et al., 2016; Piasecka et al., 2018; Quach et al., 2016; Schmiedel et al., 2018; Ye et al., 2018). These studies have identified regulatory variants related to immune activation that act in *cis* or in *trans* (e.g., master regulators detected at *IFNB1*, *IRF2*, *TLR1* or *CR1*), which are generally cell context-dependent, and have revealed major overlaps between such variants and GWAS hits (see, for example, (Fairfax et al., 2014; Kim-Hellmuth et al., 2017; Lee et al., 2014; Pala et al., 2017; Piasecka et al., 2018). These findings suggest that regulatory variation may underlie genetic associations with complex organismal phenotypes, such as infectious or autoimmune diseases (Wang et al., 2018).

Some of these studies have focused on ancestry-related variation to investigate the extent and nature of population differences in immune responses (Nédelec et al., 2016; Quach et al., 2016). Marked differences in transcriptional responses have been detected between individuals of African and European descent, African ancestry generally being associated

with a stronger inflammatory response. Up to 50% of ancestry differences are explained by *cis*-regulatory variants (Sanz et al., 2018). In a few, other cases, a single genetic variant is sufficient to explain major ancestry-related differences in gene expression. For example, the *trans*-eQTLs detected at *TLR1* or *IRF2*, which are highly differentiated between Africans and Europeans, probably account for population differences in responses to the pathogens sensed by TLR1 (such as *Escherichia coli* or BCG) or to treatment with IFNγ (Fairfax et al., 2014; Piasecka et al., 2018; Quach et al., 2016).

There is also growing evidence to suggest that natural selection has contributed to the differences in immune responses across populations, with immune-related eQTLs, both at the basal state and after immune activation or infection, enriched in signals of recent positive selection (Kim-Hellmuth et al., 2017; Nédelec et al., 2016; Quach et al., 2016). This has made it possible to identify immunological processes that may have provided a selective advantage to particular human populations, such a decrease in *TLR1*-mediated inflammation in Europeans (Quach et al., 2016) and other immune phenotypes (Sanz et al., 2018). In addition, high levels of Neanderthal ancestry have been detected among regulatory variants (Nédelec et al., 2016; Quach et al., 2016), contributing further to the diversification of transcriptional responses to infection in human populations, particularly in response to viruses (Enard and Petrov, 2018; Quach et al., 2016).

Transcriptional responses to infection can also be affected by non-genetic, intrinsic factors. For example, a recent study on transcriptional responses to bacterial, viral and fungal infections in whole-blood samples from 1,000 individuals of European descent — stratified by age and sex — revealed that ~10% of immune response variance can be accounted for by the additive effects of common genetic variants, and that sex and age account for ~5% of the total variance (Piasecka et al., 2018). However, most of these studies focused on variations of *immune gene expression* as a proxy for variations of the *immune response*. Other immune phenotypes, such as protein production or circulating immune cell frequencies, are increasingly being used to broaden our view of the various factors driving variation in immune responses (see, for example, Brodin et al., 2015; Li et al., 2016; Orru et

al., 2013; Patin et al., 2018; Roederer et al., 2015; Ter Horst et al., 2016). These systems immunology studies have been extensively reviewed elsewhere (Brodin and Davis, 2017; Liston et al., 2016), and are therefore not reviewed in detail here. Briefly, variations in the proportions of adaptive cell types have been shown to be driven mostly by non-genetic factors, such as age, sex, cytomegalovirus infection or smoking status, whereas variations in the proportions of innate immune cell types has been found to be primarily driven by host genetics (Brodin et al., 2015; Patin et al., 2018). Furthermore, epigenetic diversity (**BOX 1**) and variation of the gut microbiome have been shown to have an impact on immune response phenotypes (Chen et al., 2016; Husquin et al., 2018; Pacis et al., 2015; Schirmer et al., 2016). Quantification of the relative impacts of heritable and non-heritable factors, such as sex, age, nutrition, chronic viral infection and even social status (**BOX 2**), on the variation of immune phenotypes is the key to understanding the thresholds above which natural variation in immune responses becomes pathogenic.

Concluding Remarks

Elucidating the contribution of natural selection to the diversity of immune response genes has become an indispensable complement to immunological approaches and clinical and epidemiological genetic studies (Casanova et al., 2013; Quintana-Murci et al., 2007). Genome-wide approaches and the development of methods for detecting natural selection have enhanced the ability of population genetics studies to identify the immune functions essential for host defense, and to distinguish them from others with a greater degree of immunological redundancy or for which variation has resulted in a selective advantage for host survival in a specific population. It has also been shown that beneficial immune variation was acquired in some cases by admixture with ancient hominins such as Neanderthals or Denisovans, and that such beneficial variation is still being acquired by admixture between modern human populations. However, many questions linking population and evolutionary genetics with human immunology remain open, including the extent to which natural

selection, at different epochs, has impacted upon organismal immune phenotypes and disease risk and how past selection against infection may have some trade-offs upon changes in environmental exposures, potentially leading to maladaptation and immunopathology. Another overarching question is the identification of the specific pathogen(s) that have exerted pressure on the human genome at different time periods, and have genuinely impacted, at least in a detectable way from a genomic perspective, the evolution of specific human populations.

Some, but not all, of these questions may find an answer in the use of ancient DNA, a promising approach to identifying the genetic and evolutionary determinants of immune response variation (Skoglund and Mathieson, 2018). The study of thousands of ancient DNA samples corresponding to time points before, during and after major events, such as the transition towards more sedentary farming-based lifestyles displayed by most human populations over the last 10,000 years, or the various outbreaks of plague, which wiped out millions of people in the last millennium, should shed light on the host and microbial factors underlying susceptibility to infectious disease and the effects of selection on these factors. Efforts should also be made to develop new methods for detecting alternative selection regimes, such as polygenic adaptation or adaptive introgression/admixture, to provide us with a more complete understanding of the selection landscape characterizing the evolution of the immune system in humans.

Population genetics studies focusing on gene expression have also revealed major differences between populations in terms of the magnitude of immune responses. Most of these differences can be attributed to genetic variants segregating at different frequencies in different populations, with some of these variants presenting signals of local adaptation (Nédelec et al., 2016; Quach et al., 2016; Sanz et al., 2018). Yet, the extent to which such population differences are the neutral product of genetic drift and demography or reflect the true contribution of natural selection in differentiating immune phenotypes, including disease-related, across human groups remain to be determined. Furthermore, despite the increasing knowledge available about the genetic and non-genetic sources of immune response

variation, a large proportion of the inter-individual immune variance remains unexplained. Future investigations considering more complex forms of genetic control are required, including the roles of epistatic interactions, rare variants, interactions with age and sex, and gene-by-environment interactions (e.g., (Castel et al., 2018; Vinuela et al., 2018).

Most of the population-level studies conducted to date have focused principally on the variation of gene expression in cosmopolitan continental populations. Systems immunology studies combining multiple demographic and physiological variables with genome-wide genetic and epigenetic data and high-dimensional immune system analyses, such as flow cytometry, mass cytometry and spectrometry, are required to determine the relative contributions of genetic, epigenetic, intrinsic and environmental factors to immune diversity (Brodin and Davis, 2017; Liston et al., 2016). These studies should also be extended to indigenous and non-cosmopolitan populations of non-European descent, to improve the representation of human genetic diversity, modes of subsistence, lifestyles, and ecologies. For example, studies of this kind in the populations from the South Pacific, who have the highest levels of combined Neanderthal and Denisovan ancestry worldwide (Sankararaman et al., 2016; Vernot et al., 2016), should make it possible to quantify not only the contribution of ancient hominins to present-day human immune variation, but also to determine the contribution of this admixture to the differences between modern populations in susceptibility to infectious, inflammatory and autoimmune disorders.

BOX 1 | Population epigenetics and immune responses

The epigenome can provide information about the interplay between the environment and the human genome (Feil and Fraga, 2011), providing insight into how we respond to environmental cues. The best-known epigenetic mark is DNA methylation, which can be affected by external factors, such as nutrition, toxic pollutants, the social environment and infectious agents, and by DNA sequence variation (i.e., methylation quantitative trait loci, meQTLs). About 20% of all inter-individual variation in DNA methylation can been attributed to genetic factors (McClay et al., 2015; van Dongen et al., 2016).

Human populations are known to display considerable genetic diversity, but their degree of epigenetic diversity has been poorly studied to date. A few studies have provided an initial assessment of the contribution of genetic factors and gene-by-environment (G×E) interactions to the variation of DNA methylation at population level (Carja et al., 2017; Fagny et al., 2015; Fraser et al., 2012; Galanter et al., 2017; Gopalan et al., 2017; Heyn et al., 2013; Moen et al., 2013). In African populations, the differences in DNA methylation levels associated with differences in lifestyle (farming *vs*. hunting and gathering) have been shown to relate mostly to developmental processes and to display strong associations with nearby genetic variants (meQTLs), which are themselves enriched in selection signals (Fagny et al., 2015). Conversely, recent changes in the habitat of human populations (forest-based *vs*. urban/rural) mostly affect the methylation of immune-related genes. These studies have improved our understanding of the impact of genetic variation and of differences in lifestyle, habitat and geography on the epigenomic landscape of human populations.

One recent study focused more specifically on how ancestry-related differences affect the variation of DNA methylation and the regulation of immune genes (Husquin et al., 2018). This study explored genome-wide differences in DNA methylation in monocytes between individuals of African and European descent. Extensive ancestry-related differences in DNA methylation were detected, mostly for functions relating to the cell periphery or the regulation of immune responses. In particular, ~70% of the sites displaying differential methylation between groups of African and European ancestry were found to be associated with an meQTL, supporting the notion that differences in DNA methylation between populations are mostly driven by DNA sequence variants (Carja et al., 2017; Fagny et al., 2015; Fraser et al., 2012; Heyn et al., 2013). The integration of genetic, DNA methylation and RNA-sequencing data for the same individuals revealed substantial causal effects of DNA methylation on gene expression, demonstrating the key role of DNA methylation in controlling the transcriptional activity of primary monocytes (Husquin et al., 2018).

BOX 2 | Social status and immune response

Social status has been identified as an important predictor of disease susceptibility and mortality risk in humans and other social mammals (Marmot, 2004). Health disparities related to social status can be explained by factors such as access to resources, the long-term effects of early-life adversity and risk behaviors (Chen and Miller, 2013), but there is also growing evidence for direct physiological effects of the social environment (Archie et al., 2012; Snyder-Mackler et al., 2016; Tung et al., 2012). For example, loneliness has been linked to high levels of proinflammatory gene expression in humans (Cole, 2014). However, experimental studies are not generally possible in humans, and most studies in this field have involved the manipulation of social rank in non-human primates, which has been shown to modify the expression of genes, particularly those involved in inflammation and immune responses (Cole, 2014; Snyder-Mackler et al., 2016; Tung et al., 2012).

Experimental manipulations of dominance rank in female rhesus macaques have been used to investigate the direct impact of social status on immune function (Snyder-Mackler et al., 2016). This study clearly showed that social position, which is associated in macaques with behaviors such as received harassment (lower ranks) and affiliative grooming (higher ranks), has a direct influence on the immune system at many different scales. First, social status has a quantitative effect on cellular composition; high-ranking females have high proportions of $CD3^+CD8^+$ cytotoxic T cells and double-positive $CD3^+CD8^+CD4^+$ T cells. Second, social rank is also associated with cell type-specific gene expression patterns. For example, the number of genes with an expression pattern correlated to dominance rank is highest in NK cells, followed by helper T cells (1676 and 284 genes, respectively). Rank has been shown to affect gene expression patterns not only in baseline conditions, but also following the exposure of cells to lipopolysaccharide (LPS), to model the response to bacterial infections. A stronger and much more inflammatory response was observed in low-ranking females. Finally, dominance rank has even been shown to polarize the response of TLR4 to LPS stimulation. LPS binding to TLR4 triggers two signaling pathways: the MyD88-dependent proinflammatory pathway and the TRIF-dependent antiviral pathway (Akira and Takeda, 2004). Low-ranking females display preferential activation of the MyD88-dependent pathway, whereas high ranking females present a preferential activation of the TRIF-dependent pathway.

The impact of social and environmental effects on gene regulation is an area of active research, but the mechanisms underlying changes in gene expression in response to environmental stimuli remain unclear. In rhesus macaques, social status has been shown to modify chromatin accessibility and immune gene expression (Snyder-Mackler et al., 2018), and, in humans, environmental conditions in early life (nutritional, microbial and psychosocial) have been shown to affect DNA methylation in adulthood, in turn modifying inflammatory responses (McDade et al., 2017). Together, these studies highlight the importance of stress-driven conditions relating to social environment in the regulatory landscape of immune genes, and, more generally in physiological responses to environmental stimuli and human health (Cole, 2014).

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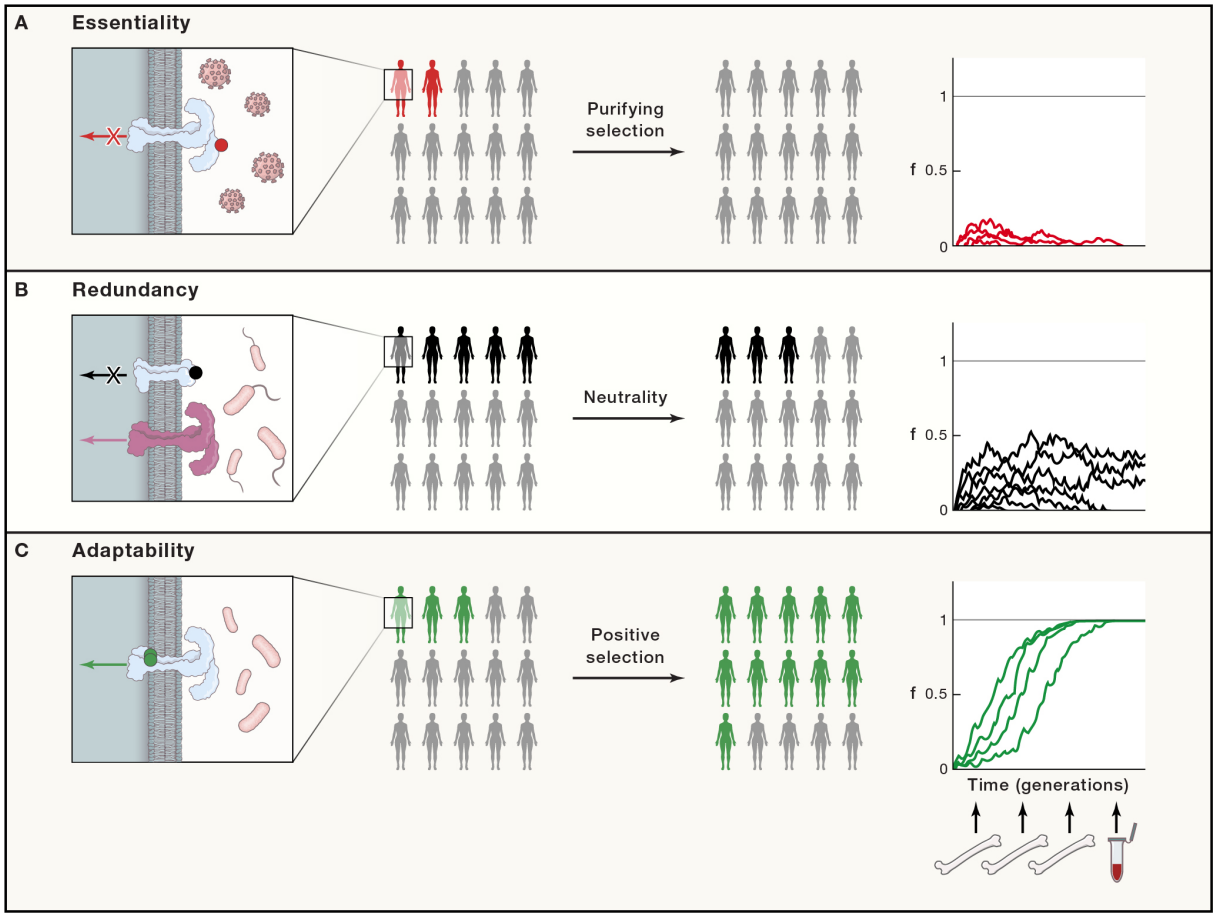

Figure 1. Understanding Immune Relevance through Population Genetics
(A) Essentiality. Mutations in genes that fulfil essential, non-redundant functions, such as TLR3 that senses nucleic acids particularly form virus, are removed from the population through purifying selection, at a pace that mainly depends of their levels of deleteriousness. (B) Redundancy. Mutations in genes that fulfil a function that is redundant from an immunological standpoint, such as TLR5 that senses flagellated bacteria, will evolve neutrally and their frequency in the human population will be mainly driven by genetic drift. (C) Adaptability. A beneficial mutation in an adaptable gene, such as TLR1 that sense microbial products at the cell surface, can improve host survival, thereby increasing in population frequency to eventually reach fixation, when it follows a classic sweep model. Studies of DNA from ancient or modern human samples increase our understanding of how these mutations, whether adaptive or not, have behaved in terms of population frequency trajectories in time.

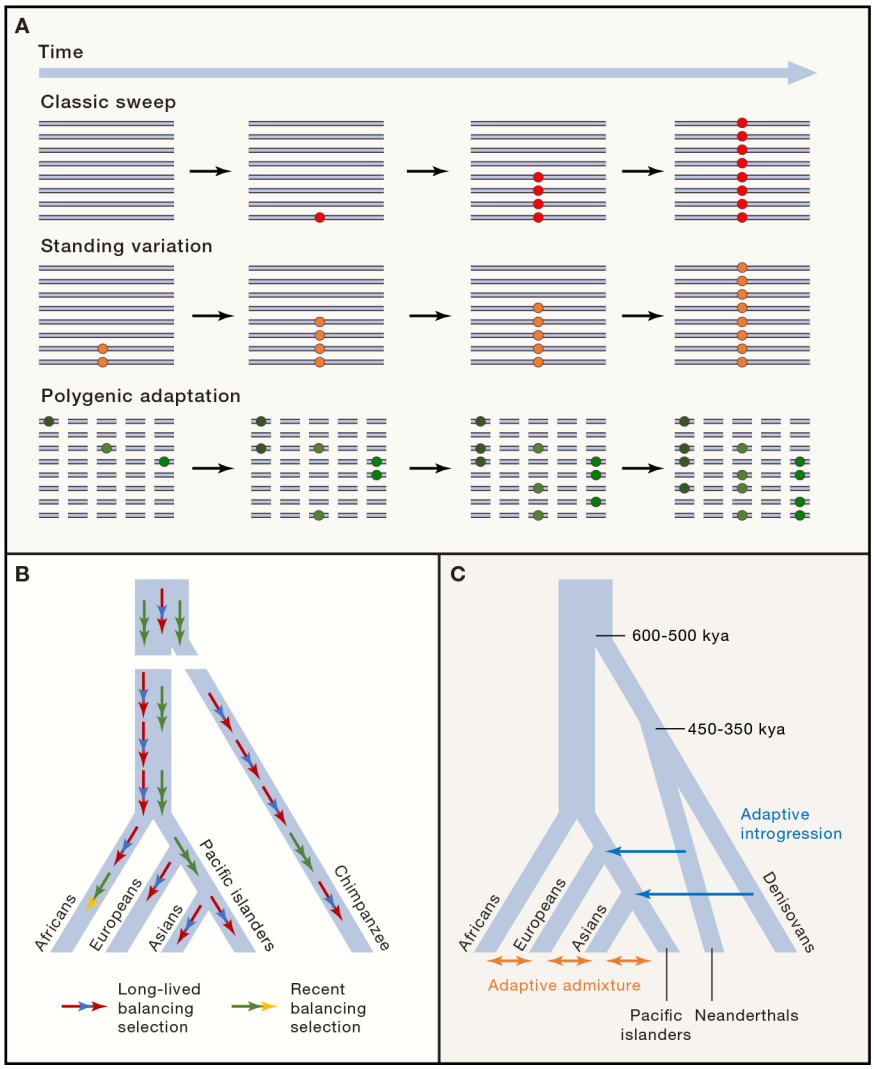

Figure 2. Different Forms of Genetic Adaptation Following Various Evolutionary Models

(A) Different models of positive selection are represented: the classic sweep, where a new mutation rapidly increases in frequency until fixation (red dots); selection on standing variation, where selection acts on a pre-existing, neutrally-evolving genetic variant in the population (orange dots); and polygenic adaptation, where multiple genetic variants located in different genomic regions simultaneously increase in frequency (green dots).

(B) Balancing selection is represented here by heterozygote advantage (colored arrows). The preservation of immune diversity can be achieved through long-lived, trans-species balancing selection (represented by red and blue arrows), in which genetic diversity is maintained at selected loci, such as the ABO blood group, over long periods of time (e.g., since the separation of the ancestors of modern humans and chimpanzees). Balancing selection can also be more recent, and population-specific (represented here by the yellow and green arrows), as illustrated by the text-book example of HbA/HbS in Africa.

(C) Beneficial genetic variation can be acquired from other species or populations through admixture. *Adaptive introgression* from archaic humans is represented by blue arrows, while *adaptive admixture* between modern human populations is represented by ochre arrows.

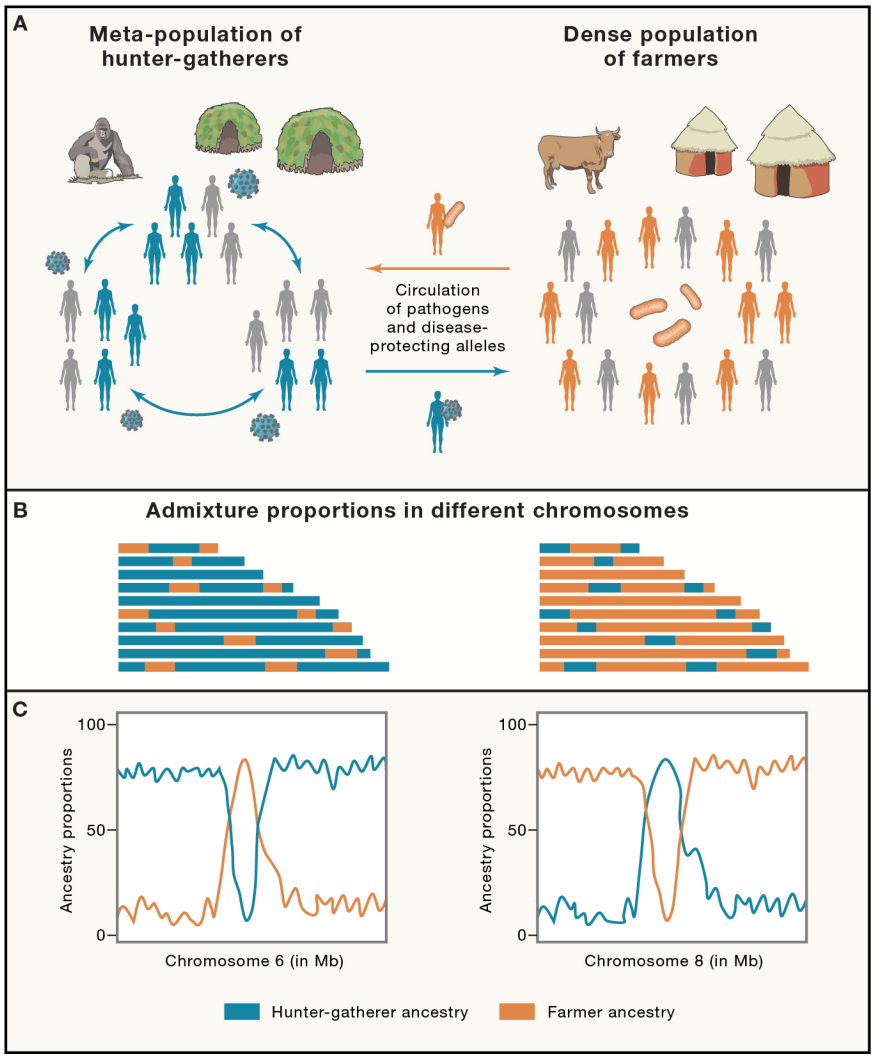

Figure 3. Genetic adaptation to pathogens of populations with different subsistence strategies can be impacted by recent admixture.

(A) A meta-population of hunter-gatherers (left) and a dense population of farmers (right) are exposed to different pathogens, in relation to their respective lifestyle and ecological settings. Socio-economic exchanges and intermarriages between groups promote the circulation of pathogens and disease-protecting alleles (blue mutation in hunter-gatherers and ochre mutation in farmers).

(B) The bi-directional gene flow between the two groups has resulted in admixed genetic ancestry. Schematic representation of admixture proportions in different chromosomes; e.g., chromosomes of hunter-gatherers (in blue) contain genomic segments that are mostly found in farmer populations (in ochre), illustrating the occurrence of admixture.

(C) A genomic scan for local excesses of ochre ancestry in hunter-gatherers (left) and blue ancestry in farmers (right) allows to identify genomic regions, here immune-related genes, that participate to host genetic adaptation, here to pathogens.