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Reconciling Mouse and Human Immunology at the Altar of Genetics

Philippe Gros[1] and Jean-Laurent Casanova[2,3]

[1]McGill University Research Center on Complex Traits, Department of Biochemistry, and Department of Human Genetics, McGill University, Montréal, Québec, Canada; email: philippe.gros@mcgill.ca

[2]Howard Hughes Medical Institute and St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA; email: casanova@rockefeller.edu

[3]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM, and University of Paris Cité, Imagine Institute and Necker Hospital for Sick Children, Paris, France

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Abstract

Immunity to infection has been extensively studied in humans and mice bearing naturally occurring or experimentally introduced germline mutations. Mouse studies are sometimes neglected by human immunologists, on the basis that mice are not humans and the infections studied are experimental and not natural. Conversely, human studies are sometimes neglected by mouse immunologists, on the basis of the uncontrolled conditions of study and small numbers of patients. However, both sides would agree that the infectious phenotypes of patients with inborn errors of immunity often differ from those of the corresponding mutant mice. Why is that? We argue that this important question is best addressed by revisiting and reinterpreting the findings of both mouse and human studies from a genetic perspective. Greater caution is required for reverse-genetics studies than for forward-genetics studies, but genetic analysis is sufficiently strong to define the studies likely to stand the test of time. Genetically robust mouse and human studies can provide invaluable complementary insights into the mechanisms of immunity to infection common and specific to these two species.

1. HUMANS OR MICE? THE IMMUNOLOGICAL DEBATE

Interindividual clinical variability during the course of infection is the general rule in humans, as recently exemplified by SARS-CoV-2 infection, for which clinical outcomes range from silent infection to lethal disease (1, 2). Defining the molecular and cellular basis of defective or protective immunity to infectious agents in humans is therefore a major endeavor, with both biological and clinical implications. The study of human immunity to infection has massively benefited from the characterization of mutant mice experimentally infected with various microorganisms. The first natural mouse mutants were identified by forward-genetics approaches in 1986, with the discovery of the *Mx* locus, which determines susceptibility or resistance to influenza virus (3, 4). Reverse-genetics approaches began the following year, in 1987, when knockout mice were generated by homologous recombination in embryonic stem cells (5, 6). Countless studies in mutant mice infected with any of a myriad of viruses, bacteria, fungi, or parasites have since been reported. These rigorous experimental studies have been extremely informative but have often been criticized by human immunologists who have called their relevance to human immunity to infection into question, on the basis that mice are too distantly related to humans, and/or that these infections are experimental as opposed to natural (7–15). Likewise, some editors and reviewers of medically oriented journals often are either reluctant to publish or have clearly stated policies not to publish results from mouse studies without an illustration or discussion of relevant human genotypes and phenotypes (16).

Conversely, studies of humans with inborn errors of immunity, which began in 1985 with the discovery of mutations of the *ADA* gene in patients with severe combined immunodeficiency (17), have long been neglected by mouse immunologists. Human genetics studies of infectious diseases can proceed only in the forward direction (reverse genetics is not possible in humans for ethical reasons). Massive amounts of immunological and clinical findings have been reported since 1946, when the clinical features of the first inborn error of immunity were reported (18), and subsequently for countless patients with any of the >450 inborn errors of immunity identified to date (19–21). However, these data are only very rarely discussed in reports on mutant mice challenged with infectious agents. Two main criticisms of human genetic studies have been expounded (22–26). First, study conditions, ranging from the patients' full genetic makeup to the nature and amount of infectious inoculum, vary considerably and are not reproducible. Second, the numbers of patients studied for any given inborn error–infectious agent pair are typically small, even sometimes limited to a single patient. Several authors have discussed the respective merits and pitfalls of immunological studies in mice and humans (7, 8, 10–15, 22–26). We aim to contribute to this discussion by highlighting the importance of the quality of the genetic study, whether performed in mice or humans. We argue that most discrepancies and controversies have arisen from an insufficiently rigorous genetic analysis of the immunological and infectious phenotypes in mice and humans. Some of the remaining discrepancies attest to insights into differences between the results of genetically sound studies of immunity to the same or related infectious agents in these two species.

2. SIMILARITIES AND DIFFERENCES IN IMMUNOLOGICAL AND INFECTIOUS PHENOTYPES

It is widely acknowledged that the general mechanisms underlying protection against infections in humans and mice are similar in terms of anatomical organization and histological layers, the major populations of leukocytes and other cell types, and their molecular sensing and response pathways. Several major basic elements of host defense discovered in mice are conserved in humans

(23). These elements include the natural protective barriers formed by epithelial cells and their products, the principal populations of circulating and resident myeloid (macrophages, monocytes, dendritic cells, and granulocytes) and lymphoid [major T and B cell subsets; innate-like T cells; and innate lymphoid cells, including natural killer (NK) cells] cells, and other tissue-specific and vascular cell types. The major molecular mechanisms involved in innate and adaptive leukocytic immunity also seem to be largely conserved in mice and humans. By contrast, the mechanisms of cell-intrinsic immunity in nonhematopoietic cells have been much less studied in both species and have rarely been found to be common to mice and humans, possibly linked to the typical species-specificity of natural pathogens (27–32). The molecular and cellular networks underlying innate and adaptive immunity have been established from studies of humans and mice, including mutant mice in particular. Some differences between humans and mice emerge from analyses of specific subsets of leukocytes, but it remains unclear to what extent these differences are driven by the use of surface markers and corresponding antibodies and which epitopes and affinities differ between mice and humans. A well-known example is provided by mucosal-associated invariant T (MAIT) cells, which are much more abundant in the blood of humans than in most strains of laboratory mice (33). Unbiased analyses of leukocytes with recently developed methods, such as single-cell RNA sequencing, CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing), and CyTOF (cytometry by time of flight), have provided clustering data suggestive of considerable similarity between the two species (34). The general principles of innate and adaptive leukocytic immunity were, thus, largely established in mice, before replication in humans, whereas the situation remains less clear for non-leukocytic immunity, especially when cell intrinsic.

Despite the many striking immunological similarities between humans and mice, there are also differences between these two species that could potentially account for the observed differences in immunity to infection (reviewed in 8, 24, 25). For example, there is only one noncanonical MHC *CD1* gene in mice, whereas there are four in humans, and there is a functional *TLR8* in humans but not in mice (see additional examples below; Section 8). These differences must be taken into account and may sometimes complicate the comparison of infections between mice and humans. But how strong are the differences between humans and mice confronted with similar infectious challenges? One striking finding is that there are more infection susceptibility phenotypes in mutant mice than in the humans bearing the corresponding mutations. Conversely, it is rare that a susceptibility seen in human patients cannot be replicated in the corresponding mutant mice. One example is provided by mutations of human genes encoding components of the complex formed by EVER1, EVER2, and CIB1, which selectively impair keratinocyte-intrinsic immunity to β human papillomaviruses (β-HPVs), but not to α- and γ-HPVs (35), as there are no known β papillomaviruses or equivalent mouse-tropic papillomaviruses (36). By contrast, susceptibilities documented in mice are often not observed in humans carrying the corresponding inborn error, suggesting different degrees of functional redundancy in certain responses between the two species, or major differences in the way susceptibility to infection manifests and is assessed in humans and mice. An example is provided by MyD88 deficiency, which disrupts signaling pathways triggered by Toll-like receptors (TLRs) and IL-1 receptors (including receptors for IL-1 cytokines and both IL-18 and IL-33) in mice and humans, underlying susceptibility to >60 viruses, bacteria, fungi, and parasites in mice but conferring only a selective predisposition to pneumococcal and staphylococcal disease in humans (37, 38). Are these differences at whole-body level a true reflection of fundamental differences in the cellular networks and molecular pathways governing host defense between the two species? Is redundancy really much greater in humans than in mice, and if so, why? We consider here some of the possible reasons for these differences, which should be taken into account when comparing results from studies in mice and humans.

3. POSSIBLE EXPLANATIONS FOR THE DIFFERENCES IN INFECTIOUS PHENOTYPES

The differences in mouse and human responses to infections may be due to various causes, acting alone or in combination (**Table 1**).

First, both the infectious agents causing disease in humans and those used to challenge mice in disease models may be natural pathogens in the corresponding species, and therefore related, but different (e.g., belonging to the same genus but each coevolving with its host species, as for *Plasmodium falciparum* in humans versus *Plasmodium chabaudi* in mice). Their natural pathogenicity solves a physiological problem, while the distant relationship between them adds a layer of evolutionary complexity. Alternatively, they may be similar (a human microbe adapted to mice) or even identical, in which case the lack of coevolution limits the physiological relevance of

Table 1 Comparison of the unique attributes of humans and mice critical for the comparative genetic analysis of response to infections

	Humans	Mice
Ancestry	Humans and chimpanzees diverged ~4 Mya. *Homo sapiens* is ~400,000 years old (divergence from Neanderthals and Denisovans)	*Mus musculus* is an ancient member of Rodentia; diverged from rats ~30 Mya
Coevolution	Different coevolution history of each species with natural pathogens that have applied individual and specific selective pressures, including leukocytes and other cell types (for example, selection of HbS in erythrocytes by malaria). Additional differences in training effects of different commensal microbiomes	
Population genetic diversity	Enormous diversity at the population level; >200,000 available whole-genome and whole-exome sequences	At population level, limited genetic diversity represented in the commercially available inbred strains of laboratory mice; only a few dozens of whole-genome and whole-exome sequences
Individual genetic diversity	Each individual is unique, with a single mixture of common and rare variants	All individuals among inbred laboratory mice are identical and homozygotes for all variants specific to that strain
Genetic analyses	Only phenotype-driven forward genetics; analysis possible in a few or even one individual	Combination of reverse and forward genetics for gene discovery in many identical individuals
Environmental conditions	Very diverse environment, including presence of pathogens and complex environmental factors that may modulate gene effects, and associated with geography, cultural, and many socioeconomic factors	Controlled environment, including absence of pathogens, and regulated temperature, nutrition, libation, and overall sanitation
Infections	Humans live in a microbial world and all infections (except live attenuated vaccines) are natural with heterogeneity of microbes	All infections are experimental, with single microbial species
Susceptibility assessment	Phenotypic measures of susceptibility are based on clinical manifestations, which are themselves modified by treatment and other interventions to improve the patient's health	Phenotypic measures of susceptibility monitored by microbial replication, survival, histopathology, and other measures at predetermined time points in multiple identical individuals
Pathogens	Have specific pathogens and disease that may be difficult to re-create in mice, even with mouse adaptation of the microbe	The mouse has rodent-specific pathogens that cause dose- and route-of-infection-dependent, unique disease and associated pathologies that mimic some but not all aspects of human infections
Immune defenses	Global similarity of leukocyte-mediated immunity, including molecular pathways involved; greater differences in non-leukocytic immunity (physical barriers, etc.)	

the experiments. In other words, the physiological relevance of experiments involving hosts and pathogens should be considered in light of evolution.

Second, infections occur in different contexts in mice and humans. They are typically experimental in mouse models and natural in humans, and this difference is likely to result in the elicitation of different immune responses, or reactions in general, both spatially and temporally. In this respect, the rare studies of natural infections in mice [norovirus, mouse cytomegalovirus (MCMV), mouse hepatitis virus (MHV)] and of experimental infections (with live attenuated vaccines, e.g., BCG) in humans (39, 40) are of particular interest, as they allow a more direct comparison of the corresponding infections in the two species.

Third, environmental factors, such as diet and nutritional status, housing conditions, and climate; intestinal and other microbiomes; history of infections (immunological memory); allergy; autoimmunity; other illnesses; and many other factors may modulate both genetic and epigenetic processes in the germline or, more commonly, in somatic genomes, particularly those of the T and B cells governing adaptive immunity (23). Mice are bred and reared in conditions designed to minimize these effects. Differences in microbial exposure in captivity or in a given strain obtained from different suppliers have sometimes led to failure to reproduce key phenotypes in independent laboratories (41–46). Indeed, adaptive immunity is particularly prone to the imprints of such influences, having been selected by convergent evolution twice in vertebrates (47) to overcome the inherent limitations of the germline, instead providing a means of continual adaption of individual organisms to countless environmental challenges. Human twin studies have confirmed that adaptive immunity is only poorly controlled by the germline (48, 49). From this angle, each geographically or ethnically defined human population, or even each individual human, is immunologically unique in space and time, including when faced with a microbial challenge; this situation is intrinsically different from that in laboratory mice.

Fourth, human patients and inbred mouse strains differ in their genetic makeup, more so for the nucleotide sequences of individual orthologous genes than for the general architecture of the genomes, although both of these factors can affect genetic studies. The impact of these differences may be greater when mutant organisms are confronted with an infectious challenge than during their baseline development and homeostasis. Many differences may affect host defense, if only because host defense involves the whole organism, including all leukocytes.

Fifth, inbred mice are not fully representative of wild mice, and differences between inbred and wild mice may have an impact on comparative studies of mice and humans (50, 51). Inbred strains are fully homozygous, and many strains carry several loss-of-function (LOF) alleles and nonsynonymous coding variants at loci involved in host defense or in genes of known or unknown function (see Section 7). Likewise, some humans born to consanguineous parents may display homozygosity in their genome, up to 6–7% for those whose parents are first cousins, and 12–13% for those whose parents are uncle and niece or aunt and nephew. Moreover, as in inbred mice, some human genes carry LOF alleles at high frequency in the general population, whether due to pseudogenization (e.g., olfactory receptors) or positive selection (e.g., *FUT2* and *APOL2*, which confer resistance to certain infections) (52). The entire set of proven and even predicted deleterious variants should therefore be taken into account when evaluating the impact of a specific allele on host defense in mice and humans.

Sixth, immunity to infection is not limited to leukocytes and their products, which have historically been the major, if not exclusive, focus of immunological studies in mice and humans. The divergence between mice and humans may be much greater for cell-intrinsic, non-leukocytic immunity than for the building of the leukocytic immune system, especially for intracellular microbes, given the coevolution between infectious agents and their target hosts (7, 53). Malaria is a case in point, with major genetic determinants of susceptibility to infection in humans being

largely restricted to proteins of erythrocytes, the major ecological niche of blood-stage *Plasmodium* (54, 55).

4. INFECTIOUS AGENTS TROPISM FOR MICE OR HUMANS

When analyzing the impact of germline mutations on vulnerability to infections, it is essential to consider whether the microbe studied has a history of natural tropism for, and coevolution with, the host. In the case of emerging infections in humans, it must be determined whether the microbe in question can or does also infect mice, naturally or experimentally. With few exceptions (e.g., inoculation of live attenuated vaccines), the vast majority of human infections occur naturally, via natural routes forged during the course of evolution at the population level. Infection may follow an accidental encounter between the subject and the microbe (e.g., inhalation of *Mycobacterium tuberculosis* upon contact with a patient), or the breach of a natural barrier if the microbe is commensal (e.g., cutaneous *Staphylococcus aureus*). When studying human pathogens that do not naturally infect mice (either in the wild or in the laboratory)—the most frequent scenario, as microbes naturally pathogenic for both species are rare—arbitrary phenotypes at the organism (e.g., survival, weight) and tissue (e.g., microbial replication, inflammation) levels must be studied and monitored at defined time points to define susceptibility, resistance, and their mechanisms. This situation results in significant variability between the experimental results obtained for different infection models and even for the same model in different laboratories. The microbe itself may be a pathogenic isolate obtained from a human patient or an adapted strain selected on the basis of mouse permissiveness to the specific strain concerned. Despite the lack of natural context and history, many pathogens can serendipitously induce similar lesions in mice and humans. These pathogens include *M. tuberculosis*, which can induce pulmonary tuberculosis following exposure to very low doses of aerosol in both species. It could nevertheless be argued that the infection of mice with *Mycobacterium microti*, a natural pathogen of rodents, would be more physiologically relevant, as *M. tuberculosis* does not naturally infect mice in the wild (56). Human-tropic members of the coxsackievirus group, such as CVB4 and CVB3, also readily infect mice, causing similar diseases in both hosts (myocarditis, meningoencephalitis, hepatitis, and pancreatitis) (57, 58). A wealth of knowledge can therefore be retrieved from experimental infections in mice with microbes that are natural pathogens in humans, despite these microbes not necessarily being natural pathogens or commensals of mice.

Nevertheless, several human pathogens are either intrinsically less virulent or even completely avirulent in mice or cause disease only in the context of specific mutations that weaken mouse immunity. For example, mice are generally resistant to infection with gram-positive bacteria, such as *Staphylococcus* and, to a lesser extent, *Streptococcus* species, for which high infectious doses are required to induce even transient infection (59–61). Likewise, productive systemic infection with human fungal pathogens, such as *Candida albicans*, is difficult to induce in mice, requiring high infectious doses in a context of complement C5a deficiency (62), and very stringent experimental conditions are also required for mucocutaneous infection (63). Several human viruses [e.g., herpes simplex virus 1 (HSV-1)] cause disease in mice, but high infectious doses and specific routes of introduction are required for the development of disease resembling that observed in humans [e.g., herpes simplex encephalitis (HSE)] (64). In yet another turn, the same infectious agent can cause vastly different diseases in mice and humans. In humans, the protozoan parasite *Leishmania donovani* causes very severe visceral leishmaniasis, whereas experimental infection in mice results in no more than a transient self-limiting infection (65). Conversely, *Salmonella enterica* Typhimurium causes a lethal systemic infection reminiscent of human typhoid fever in mice, but only causes self-limiting gastroenteritis in most humans (except those with specific immunodeficiencies) (66).

Finally, other human pathogens, such as the H3N2 and H1N1 influenza viruses, must undergo serial passages in mice for the purpose of adaptation or to increase virulence, and this may involve changes that are not relevant to the human infection (67). Overall, the infectious agents used in mouse studies may be very different from their human counterparts, either intrinsically or in terms of the disease they induce, and caution is therefore required when comparing infectious phenotypes.

5. NATURAL AND EXPERIMENTAL LIVING CONDITIONS

One major difference between experimental and natural infections concerns the environment in which mice and humans live. Mice are kept in controlled conditions in animal facilities with environments differing in their degree of microbe exclusion, ranging from specific-pathogen-free (SPF) to completely sterile (germ-free, gnotobiotic). They do not eat what they would eat in the wild, and coinfection with other pathogens is prevented for the sake of rigor. Indeed, the physical conditions in animal facilities differ in several ways from conditions in the wild, with significant effects on immune system development and function (68): (*a*) An ambient temperature of 20–25°C is comfortable for humans but low for mice, necessitating increases in metabolic activity (69). (*b*) Light/dark cycles and associated circadian rhythms affect the leukocyte populations and response to infection (70, 71). (*c*) Exercise levels are lower and overall stress levels are higher in captivity (72). (*d*) The intestinal and cutaneous microbiomes of laboratory mice are relatively stable, depending on their diet and other housing conditions. Mice are coprophagic, and an increasing number of studies have documented the important impact of the mucosal surface microflora on myeloid and lymphoid cell development and response to infections, inflammation, and autoimmunity (73, 74). (*e*) Laboratory mice used in immunological studies tend to be young (8–12 weeks of age) and have an adaptive immune repertoire that is largely naive, with fewer memory T and B lymphocytes than adult humans.

Mice have been kept for decades in SPF conditions. The exclusion of natural pathogens from the immune history of these animals (prevention of bystander infections) leads to differences in the training of the immune system and associated baseline immune phenotypes relative to wild mice or mice from pet stores. These differences may concern the numbers and functions of CD4 and $CD8^+$ T cells, the type I interferon response, and several other immune pathways (22, 23, 26). The presence or absence of such infections has profound effects on the expression of mutant immune phenotypes. For example, (*a*) *Hoil1*$^{-/-}$ mice (*Rbck*) are susceptible to *Listeria* infection but are protected against this infection when chronically infected with gammaherpesvirus 68 (75); (*b*) human *ATG16L1* variants contribute to inflammatory bowel disease; however, *Atg16l1*$^{-/-}$ mouse mutants are not susceptible to chemically induced colitis unless infected with norovirus (46); and (*c*) chronic infection with MHV modulates susceptibility to *Salmonella* infection (76), whereas latent infection with MCMV modulates susceptibility to *Listeria monocytogenes* and *Yersinia pestis* infections (77). A list of mouse pathogens excluded from mouse colonies and their impacts on the immune system is available (23). These differences have led to the concept of naturalizing mouse models to improve their relevance for immunological studies and, ultimately, to facilitate comparisons between mice and humans (68, 78) (see Section 14). Profound changes in the immune system have been observed in dirty mice (generated by housing mice together, fecal transplantation, surrogate fostering, and even rewilding, with laboratory mice placed outdoors) (22), including a loss of the *Stat6*$^{-/-}$ (79) and *Nod2*$^{-/-}$ (79) phenotypes seen in laboratory mice bred and tested under clean conditions. Hence, environmental factors (which differ between mice and humans) should be taken into account when interpreting and comparing human and mouse data.

6. NATURAL VERSUS EXPERIMENTAL INFECTIONS

Another important difference between infections in humans and mice is the process by which the pathogen is introduced (natural in humans, experimental in mice), including important variables, such as dose, route, coinfections, and the environmental conditions of sanitation and immunity in which infections occur. Natural infections in laboratory mice have rarely been studied (23). Instead, experimental infections are much more frequently studied, and these may involve variable and sometimes high doses of a single infectious agent. Moreover, the infectious agent is introduced as a single homogeneous and synchronous bolus, and these conditions are not representative of most natural infections. Furthermore, the subcutaneous, intramuscular, intravenous, intraperitoneal, retro-orbital, or other nonnatural routes bypass key natural anatomical protective barriers. Finally, for certain vectorborne infections (e.g., underlying malaria, trypanosomiasis, leishmaniasis, dengue, yellow fever, and many other infectious diseases), the stage in which the pathogen is carried by the natural insect vector (and the associated life-cycle forms of the pathogen) is generally bypassed in common experimental mouse models of these infections. By contrast, the vast majority of human infections take place in natural situations, possibly involving low doses and repeated exposure, probably involving coinfection with other microbes, and with natural routes of infection (e.g., vector, aerosol, ingestion, skin lesion). Moreover, immune responses in humans may occur in individuals of any age, from neonates to centenarians. They may therefore be influenced by several factors not reproduced in mouse models, including prior exposure to the same or a closely related pathogen or antigenically related microbes (e.g., seasonal influenza viruses from previous years), which may affect adaptive immunity, or even exposure to unrelated microbes, which may activate innate immunity (77). Furthermore, active immunization through vaccination programs and prior treatments with antimicrobial drugs may also have an impact.

However, not all human infections are natural. For example, inoculation with live attenuated viral or bacterial vaccines can provide unique insight into the similarities and differences in immunity between humans and mice. Live attenuated vaccines are considered safe in the vast majority of humans, but each one has eventually been found to cause clinical disease in at least a few, typically individuals with rare inborn errors of immunity (40, 80, 81). For example, *Mycobacterium bovis* BCG can cause clinical disease in individuals with inborn errors preventing the development of autologous T cells [a condition known as severe combined immunodeficiency (SCID), which underlies various infections] or selectively disrupting IFN-γ immunity [Mendelian susceptibility to mycobacterial disease (MSMD)] (**Figure 1**; see below). Live poliovirus vaccine typically causes disease in people with inborn errors of B cells. Adverse reactions to live attenuated viral vaccines, such as the measles-mumps-rubella (MMR) and yellow fever virus (YFV) 17D vaccines, can be caused by inborn errors of type I interferons or their autoimmune phenocopies (82). These exceptions aside, differences between natural infections in humans and experimental infections in mice, together with differences between the natural and experimental conditions of life in humans and mice before infection, are likely to influence immune and leukocytic responses. They may globally impact the reaction of the whole host, both qualitatively and quantitatively, with different effects on pathogenesis. Finally, as discussed below in greater depth, the great diversity of human beings makes each infection unique, as each person may have inherent genetic determinants, a singular history of other infections, or a specific treatment that weakens immunity. Overall, infections with a given pathogen occur in very different contexts in humans and mice.

7. MICE AND HUMANS ARE GENETICALLY DISTANT COUSINS

Both mice and humans are mammalian vertebrates. They are distant relatives that diverged from a common ancestor ~95 Mya, whereas rats and mice diverged much more recently (~25–30 Mya)

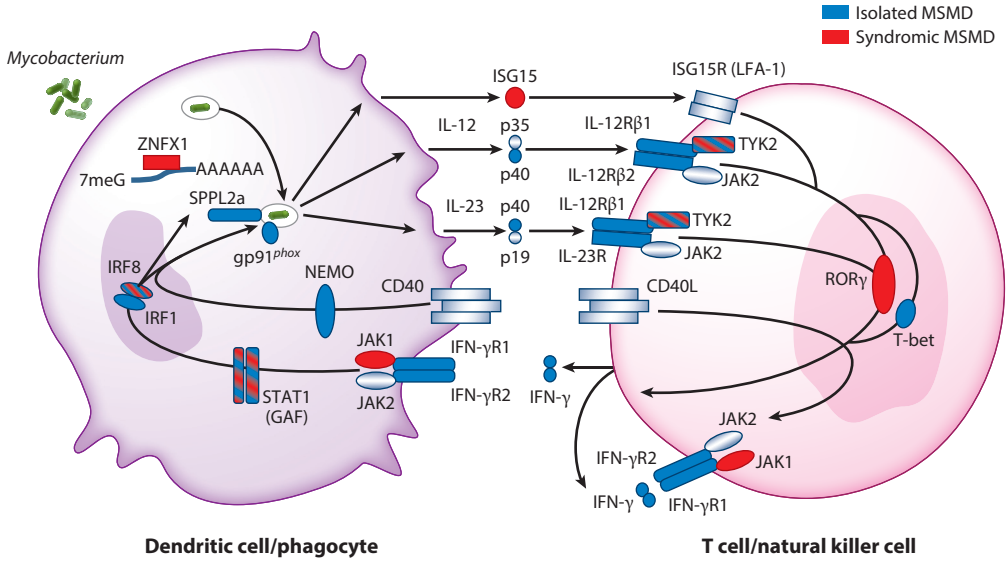

Figure 1

Human genes in which mutations convey susceptibility to infection with mycobacteria. Genes expressed in myeloid cells (dendritic cells, phagocytes) and in lymphoid cells (T cells, natural killer cells) in which mutations have been identified in patients with isolated MSMD (Mendelian susceptibility to mycobacterial diseases) and BCG-osis following perinatal vaccination with BCG (*blue*), or in patients with syndromic MSMD and BCG-osis concomitant to other infections (*red*). The clustering of genes and mutations highlights the critical role of the IL-12–IFN-γ signaling loop in protection against mycobacterial diseases.

(83, 84) and humans and chimpanzees diverged more recently still (~4 Mya) (85). By contrast, the common ancestor of fungi/plants and animals is estimated to have lived ~500–600 Mya (86), that of vertebrates and invertebrates ~530 Mya (87), and that of jawed fish and jawless fish ~500 Mya (88). Our own modern species, *Homo sapiens*, is estimated to be about 400,000 years old (beginning from the time at which it diverged from Neanderthals and Denisovans), whereas *Mus musculus* emerged following an older obscure subspeciation event in Rodentia >56 Mya (84, 89). Rodents are probably among the most ancient mammals in existence, having been present on Earth since the radiation of small mammals during the era of the dinosaurs. Larger mammals emerged only later, once the dinosaurs were extinct. Humans emerged in East Africa, whereas mice emerged in Central Asia (90). The first encounter between modern humans and modern mice is, therefore, thought to have occurred no earlier than 400,000 years ago. By comparison, microbial life dates back ~3.5 billion years, with archaea and bacteria existing in near isolation for about 1 billion years. Another billion years later, unicellular eukaryotes emerged, but it was not until the last billion years that multicellular eukaryotes emerged and survived several rounds of extinction. In other words, mice and humans, like all their ancestors, emerged and evolved in an environment that had long been populated by viruses and unicellular prokaryotes and eukaryotes. The pathogens and commensals in both mice and humans are the heirs of the first living organisms on Earth. Microbes have exerted enormous selection pressures on multicellular eukaryotes, including mice, humans, and their ancestors. These selective pressures have shaped natural barriers, the various layers of intrinsic, innate, and adaptive immunity protecting humans and mice from the preexisting microbes that surround them. Geography has also contributed to these historical constraints. Natural infections in humans and mice clearly depend on the presence of the corresponding pathogens in the geographic environment in which they live. The presence or absence of such pathogens or of their

ancestors in the past affected the evolutionary context in which the immunity of these populations developed in their ancestors and the shaping of modern-day immunity, in both species (53).

Mice have 20 pairs of acrocentric chromosomes (19 autosomes and X/Y), whereas humans have 23 pairs (22 autosomes and X/Y) of mostly metacentric chromosomes. The chromosomes of the two species display >220 regions of synteny (linkage groups; chromosomal segments in which the genes and their order are preserved) covering >90% of each of the two genomes (91). The protein-coding genes of the mouse and human genomes are more than 98% identical overall. However, much more is known about the level of intraspecies, interindividual genetic variability—which is predicted to be a key determinant of interindividual clinical variability during the course of infection—in humans than in mice, with more than one million genomes and more than one million exomes from human individuals of several ancestries sequenced to date, versus only a few dozen genomes from inbred strains of mice. Overall sequence conservation is low for the non-protein-coding portions of the genome (<50% nucleotide identity) but higher for the protein-coding portions of the genome, at about 70–75% amino acid similarity (identity and conservative substitutions between orthologous proteins) (92). These numbers are influenced by methodologies used for alignment and the annotation tools used for genome and protein sequences. Species-specific genes account for less than 2% of the total number of ~20,000–22,000 protein-coding genes present in the mouse and human genomes (ENSEMBL; 22,519 versus 20,448, respectively). This has led to the conclusion that there are very few genetic differences between mice and humans. However, the 1–2% of genes that are species-specific mostly belong to multigene families, with expansion and drift rates that differ between humans and mice, resulting in variable accumulation of pseudogenes in these clusters. This differential expansion appears to be due, in part, to differences in selective pressure, the corresponding genes being involved in functions such as olfaction, reproduction, lactation, and, interestingly, protective immunity (92). For example, the olfactory receptor (OR) gene clusters in mice are larger and contain fewer pseudogenes (20% pseudogenes) than those in humans (60% pseudogenes) (93, 94), suggesting better olfactory performance in mice and gradual loss of olfaction capacity in primates, which might have increasingly relied on their other senses to survive.

8. THE MOUSE AND HUMAN GENOMES DIFFER AT IMMUNOLOGICAL LOCI

There has been a rapid divergence of genes associated with the hematopoietic immune system in certain gene clusters, possibly due to microbial selective pressure (53, 92). For example, mice have an extensive family of 8 to 12 *Ly49* natural killer receptors (activating and inhibitory receptors interacting with MHC-I molecules), depending on the mouse strain, but only the proto-*Ly49* gene appears to be preserved in the human lineage, as a solitary pseudogene (95, 96). Some virus-derived proteins [e.g., m157 from cytomegalovirus (CMV)] are recognized in association with various Ly49 proteins in mice to produce activating or inhibitory signals in NK cells, thereby affecting the host response to infection. Conversely, humans have 6 or more killer cell immunoglobulin-like receptors (KIRs), whereas mice have none (97). These human receptors are structurally unrelated to the Ly49 receptors in mice, but they serve the same function (98). Other examples of immunity gene clusters diverging between humans and mice include those for the Naip family of receptors for bacterial flagellin (2 in humans and up to 6 in mice) (99), clusters of defensin antimicrobial genes, WAP domain antimicrobial genes and type A ribonuclease genes (100), genes encoding the MHC-Ib region (101), the OAS family (102), genes for the noncanonical MHC molecule CD1 (CD1a in mice, but CD1a–d in humans) (103), and several other so-called immunity genes (25, 92). Another example is provided by the type I IFN gene family and associated clusters, which

comprise 17 bona fide genes and 1 pseudogene in humans, but up to 30 genes (including *limitin* genes) and 1 pseudogene in mice (104–107).

It should also be borne in mind that each species has at least 500 genes not found in the other, including known and potential immunity genes, and that the levels of identity between orthologous protein-coding genes vary considerably between loci. Moreover, the non-protein-coding regions of the two genomes diverge considerably. Little is known about the differences between mice and humans for non-protein-coding transcribed genes/cistrons, including miRNA, snoRNA (small nucleolar RNA), and lncRNA. Several miRNAs have clearly established roles in regulating immune function (108–110), but the degree of structural (sequence) and functional conservation between humans and mice remains unclear.

Finally, host defense against infection is not restricted to the general leukocytic immune system, including circulating and resident leukocytes, as it also involves most, if not all, of the more than 400 discernable non-leukocytic cell types present in mice or humans. These cells and the corresponding genes may make considerably different contributions, cell intrinsic or extrinsic, to infection control in humans and mice. Such differences may be highly significant, as the contributions of non-leukocytic cell types to protection against infection may be subject to evolutionary imprints from natural pathogens in both species that are at least as strong as those imposed on the innate immunity mediated by professional leukocytes. Classic examples include mutations of the human *FUT2* gene conferring protection against intestinal norovirus infections (111); mutations of *DARC* and *G6PD* that prevent *Plasmodium vivax* infection in erythrocytes or reduce replication of this microbe in these cells (112); the sickle cell trait (heterozygosity for the HbS variant), which confers relative resistance to severe forms of *P. falciparum* malaria (54); and several other genetically encoded malaria-resistance traits phenotypically expressed by erythrocytes or erythrocyte precursors (113–115). Thus, despite the close relationship between the genomes of humans and mice, significant divergence exists, particularly within clusters of genes associated with immunity to infections.

9. INBRED MICE CARRY VARIOUS DELETERIOUS MUTATIONS

The mice used in biomedical research (*Mus musculus musculus*) are not wild-type, outbred mice in which free recombination and exchange of allelic variants create homogeneity and heterozygosity at the population level, much as observed in human populations. Instead, mice are available as a large number (~400) of independently derived fully inbred strains maintained by commercial suppliers through systematic brother-sister mating. Some of the oldest inbred strains (East Asian and European "fancy" mice) were developed many decades, or even centuries, ago from feral mice and were initially selected for breeding performance, attractive appearance (coat color), and interesting behavioral phenotypes (fancy mice) (90, 116, 117). Over the years, additional strains were developed, through either recapture from the wild or independent derivation from existing stocks (118). The mouse strains used in biomedical research are fully inbred and homozygous at all loci. However, the individual inbred strains are significantly different from one another. They carry a subset of naturally occurring genetic variants fixed to homozygosity and physically linked on defined haplotypes (117). C57BL/6J (B6) is the mouse strain most widely used in biomedical research, including for the production of knockout mice. A recent complete genome sequence analysis of the 36 most widely used inbred strains showed that, as a group, these mouse strains carry a number of protein-coding gene mutations predicted to be deleterious or even LOF. Relative to the other 35 inbred strains analyzed, the B6 genome harbors 38 genes with the gain of a premature stop codon, 65 genes with the loss of a natural stop codon, and 261 other genes with missense variants predicted to be highly detrimental (PROVEAN scores > 5.0) (92). There are also many other in-frame variants that are possibly deleterious and common to B6 and other inbred strains.

An analysis of these lists with the MGI Mammalian Phenotype browser and associated ontology annotation revealed that among these, 5 gain-of-stop (GOS) genes, 13 loss-of-stop (LOS) genes, and 156 deleterious variants (DVs) have immune function and host defense annotations. These genes are involved in regulating the number, differentiation, function, and activation of myeloid and lymphoid cells; the sensing and processing of microbial products, cytokines/chemokines, and their receptors; downstream intracellular signaling cascades; microbicidal functions; cell-cell interactions; susceptibility to infections; and several other functions. Genes harboring GOS variants, LOS variants, or structural variants (SVs) include those encoding CD160, CD180, CD48, CD3EAP, C4b, IRAK4, KLRA2, FCGR2B, FCGR3, FCGR4, IL31RA, ITGA6, JAK3, LRRK1, NF1, NLRC5, NLRP12, NLRP9B, NOD2, SLC11A1, TAP1/TAP2, STAT2, TLR9, TNFRSF1B, and TYK2. Although most of these variants are not specific to B6, a subset of them are. Thus, B6 is a mouse of uncertain genetic status with respect to immune genes, and several of its pathways may already be altered before the production and testing of knockouts. Moreover, the function of most of these genes remains largely unknown, and many genes were never before implicated in host defense. In fact, genes governing non–hematopoietic cell immunity may be mutated in these mice, because of the poor evolutionary advantage provided by these genes in pathogen-free laboratory facilities.

Humans also carry a number of LOF variants of key components of immune pathways that are different from natural deleterious variants in mice but that may provide information about human-specific and individual responses to infection (52). These variants are rarely common to all humans or all human ancestries. Instead, they are mostly specific to certain kindreds or individuals. Any mutation of interest, whether in humans or mice, should be considered in the context of all variants predicted or shown to be deleterious before any particular phenotype is assigned to it. Additional genetic studies in live animals (genetic complementation, allelic series), humans (genetic homogeneity across ancestries), or cultured primary cells (complementation or knockout) are crucial to validate the causal relationship between genotype and phenotype.

10. PUBLIC MOUSE VARIANTS: FORWARD AND REVERSE GENETICS

Several variants predicted to be deleterious are specific to a single inbred mouse strain (private), but many others appear to be ancestral and are present in several strains (public). Public variants are fixed in conserved haplotypes in inbred strains. However, their assortment (on different chromosomes) differs between individual strains, and their combined actions determine interstrain differences in response to infections with viral, bacterial, fungal, and parasitic pathogens. These deleterious variants and their associated haplotypes may be confounding and should be taken into account when interpreting reverse genetics experiments. The distribution of such haplotypes and associated deleterious mutations in inbred strains must also be taken into account when comparing immune responses in humans with those in inbred mouse strains with deletions at a specific locus. For example, *Casp1*$^{-/-}$ knockout mice are also homozygous for a natural LOF variant of *Casp11* and therefore have a double deficiency. The two genes concerned are tightly linked, and no segregation of the two events was observed when the 129 knockout was backcrossed onto the B6 background; several phenotypes of the *Casp1*$^{-/-}$ mutation were therefore unwittingly attributed to this gene only (119, 120). This problem does not arise in forward genetics studies, in which the candidate loci are identified by means of genome-wide linkage and sequencing.

On the other hand, certain deleterious germline variants in inbred strains of mice have proved very useful for forward genetics studies. Indeed, the genetic study of infectious phenotypes has revealed that these private and public variants and associated haplotypes can govern key mechanisms of protection against infections. Examples of functional haplotypes with binary

distributions in inbred strains controlling differential susceptibility to infections include the *Bcg/Nramp1* (Slc11a1) locus on Chr. 1, which confers different degrees of susceptibility to infections with *Mycobacterium*, *Salmonella*, and *Leishmania* (121), and the haplotypes on Chr. 2 (*Hc* locus; C5a) conferring differential susceptibility to *C. albicans* (62) and to *Plasmodium berghei* (122). The SMJ mouse strain has an additional major specific locus regulating *C. albicans* infection (123). Haplotypes at the *Cmv1* locus on Chr. 6 (*Ly49* cluster) determine susceptibility and host response to CMV (98). A Chr.1 locus and associated haplotype control susceptibility to blood stage malaria (124–126). Another classic example is the LOF mutation of *Mx*, a key interferon-stimulated gene (ISG) in defense against influenza viruses that is present in almost all inbred mice. In this context of constitutive absence, it is difficult to study the impact of other type I IFN–related genes (3, 127). Unique phenotypes (different from those in the parental strains) can also be generated by epistatic interactions between public variant–bearing haplotypes (often revealed in F2 mouse populations). For example, an epistatic interaction was found to occur between allelic combinations at a locus on Chr. 4 (*Brr6*), and at a locus on Chr. 1 (*Brr7*) in mice produced by an (B6 × 129S1/SvImJ) F2 cross that display high resistance to cerebral malaria whereas both the parental strains are susceptible (128). The Chr. 4 locus contains the type I interferon gene cluster, suggesting that its epistatic modulation may have a significant impact on susceptibility to many infections in such F2 mice.

11. PRIVATE MOUSE VARIANTS UNDERLYING INFECTIOUS PHENOTYPES

Strain-specific private variants (new mutations) may arise either during the initial derivation of an inbred strain or when new colonies of the same strain are generated and maintained as independent colonies. These variants may be confounding in reverse genetics studies, but their characterization has proved enormously valuable following their identification by forward genetics. Some of the best-known examples include the discovery of *Tlr4* as a sensor of LPS and mutated (*Lps* locus) in C3H/HeJ mouse strain (129). Another early example is the *Xid* mutation of the *Btk* gene (Bruton tyrosine kinase), which causes a severe defect of B cell development (X-linked agammaglobulinemia) specific to the CBA/N strain (130, 131). The *Lps* and *Xid* mutations cause susceptibility to *Salmonella* despite the presence of naturally protective *Bcg/Nramp1* Chr.1 haplotypes in the two strains concerned. Other examples include the *Scurfy* mouse, which has an X-linked *Foxp3* mutation. Studies of this mouse revealed the molecular basis of regulatory T cells (132), and the human ortholog of the affected gene was subsequently shown to be mutated in patients with IPEX (immunodysregulation, polyendocrinopathy, enteropathy X-linked) syndrome (133, 134). Even substrains of commonly used B6 mice generated in different locations may also carry substrain-specific mutations, such as the *Dock2* mutation, which affects the numbers of $CD8^+$ T cells and splenic marginal B cells in sublines of C57BL/6NHsd mice (135).

Even in mixed genetic backgrounds, such as that of recombinant inbred or recombinant congenic strains, such mutations may arise and convey novel infection phenotypes different from those of the two parents. The BXH2 recombinant inbred strain, unlike its parental strains (B6 and C3H/HeJ), is extremely susceptible to mycobacterial infections, despite the presence of naturally protective *Bcg/Nramp1* Chr.1 haplotypes (136). This phenotype is caused by a mutation of *Irf8* (137) that arose during the derivation of this strain and that impairs dendritic cell development (138). Similar dendritic cell defects in humans were subsequently found to be caused by IRF8 deficiency, which also conferred susceptibility to infections with mycobacteria and other microbes (139–141). A related case is the BXD8 mouse strain, which carries the protective B6 haplotype ($Cmv1^r$) but is susceptible to MCMV infection due to a deletion in Ly49h. Another example is provided by the recombinant congenic strain AcB55 (B6 and A/J parents), which is extremely

resistant to malaria due to a mutation of the gene encoding erythrocyte pyruvate kinase (*Pklr*^{I90N}), which was fixed during the derivation of a few such AcB strains (*Char 4* locus) (142, 143). However, another AcB strain, AcB61, bears protective *Pklr*^{I90N} alleles but is susceptible to malaria due to independent inactivation of the *Vnn3* gene (*Char9* locus), a lesion acquired during the derivation of this strain (144). Finally, Pklr-deficient AcB62 mice carry yet another modifier locus (*Char10*) on chromosome 9 that modulates the penetrance and expressivity of protective *Pklr*^{I90N} alleles through compensatory erythropoiesis (145). These results indicate that, even within the 8- to 10-year breeding program used to derive the AcB/BcA strain set (124), several of the strains have acquired and fixed novel mutations with a profound impact on susceptibility to malaria.

12. FORWARD GENETICS BY ENU MUTAGENESIS

Inbred mice, studied alone or in combination, do not accurately reproduce the genetic diversity of human populations and of rare human patients. However, their study by means of forward genetics has been both rigorous and fruitful. Most loci discovered by forward genetics in mice have been found to be mutated in humans with similar phenotypes. Similarly, most of the loci discovered in humans by forward genetics studies, such as the *AIRE*, *BTK*, and *IRF8* genes, have also been found to underlie a similar phenotype in the corresponding mutant animals. This success led several laboratories to develop research programs aiming to generate new random variants by *N*-ethyl-*N*-nitrosourea (ENU) chemical mutagenesis, and then to perform various forward immunological or infectious screens to identify morbid genes by whole-exome or whole-genome sequencing. This approach has been extremely successful for identifying mutations and the associated genes playing crucial roles in response to different infectious pathogens and associated immune response pathways. In their work monitoring changes in peak parasitemia following infection with *Plasmodium chabaudi* coupled to a secondary screen for hematological/erythroid phenotypes, Foote and colleagues (146) identified a number of genes in which variants affect the response to blood-stage malaria, including *Ank1* (ankyrin-1), *Sptb* (spectrin-b), *Tfrc* (transferin receptor 1), and *Ampd3* (adenosine monophosphate deaminase). These mutations affect metabolism of the erythrocytes, the ecological niche of blood-stage infection. In parallel small-scale studies, we have identified mutations that blunt lethal neuroinflammation and protect mutant mice against cerebral malaria induced by *P. berghei*. These mutations affect the number and functions of T cells (*Zbtb7b*, *Jak3*, *Themis*) (147–149), the mobility of myeloid and lymphoid cells (*Ccdc88b*, *Rasal3*, *Arhgef2*) (150, 151), and the type I interferon response and RIG-I signaling (*Usp15*, *Trim25*) (152).

This approach was pioneered and has also been used, at a much larger scale, by the group of Beutler (153, 154), who has successfully mapped a number of genes (with allelic series) and associated pathways involved in several immune functions, including but not limited to the innate recognition of pathogens, the innate response to pathogen-derived products, and the response to MCMV. The *Mutagenetix* database (**https://mutagenetix.utsouthwestern.edu**) contains thousands of ENU-induced mutants identified by mapping and sequencing (155, 156). It includes 4,694 mutations of 2,310 genes that have been declared causal for 22,655 phenotypes. More than 90,000 additional mutations of 15,330 genes have also been cataloged and are considered to have a high likelihood of causing phenotypes. In this remarkable resource, all mutations and associated strains can be retrieved and obtained for screening against any phenotype. A similar ENU mutational database developed by Goodnow and his team now contains over 1,900 mutant strains with mutations of 1,667 genes with associated annotations and is accessible to the research community (**https://pb.apf.edu.au**). This group also implemented forward genetics screens for mutations affecting self-tolerance, autoantibody production, and autoimmunity, leading to the discovery of critical genes involved in these pathways (those encoding CARD11, ROQUIN1, HNRNPLL,

THEMIS, DOCK8, ATP11C, SPPL2A, ZFP318, GSDMD and ETAA1) (157). Overall, ENU mutagenesis has proved extremely valuable for the forward genetics discovery of genes and pathways regulating normal immune function, alterations of which result in disease (when coupled with phenotype-driven screens) in a fixed genetic background that alleviates or eliminates limitations of the genetic background effects described above. Furthermore, its combination with ingenious breeding schemes and genome sequencing has provided the research community with a vast array of ready-made mutants with mutations in desired genes annotated in searchable databases.

13. LIMITATIONS OF REVERSE GENETICS IN MICE

The phenotype-driven approach in mice has also revealed an oligogenic or polygenic basis for the host response to infections in many cases. In such studies, the loci identified were ranked according to their relative genetic contribution to the phenotype in informative populations of mice in which the mapped loci segregated (for example, 143, 158). Positional candidate genes at these loci can be formally tested individually, in engineered knockout mice. However, in this case, the knockout is not studied in the context of the rest of the genome, and the presence or absence of a phenotype (in comparisons of knockout and wild-type littermates) may reflect the oligogenic nature of the initial trait and/or may be a consequence of compensatory effects at other loci that blur the results. It is possible to overcome these pitfalls by determining the relative contribution of each locus in congenic mouse strains in which the chromosomal segment responsible for the effect has been introgressed into a different genetic background, but this is very rarely done. When such an approach is used, the contribution of the locus to the total phenotypic variance (estimated initially from the strength of linkage and the effect in segregating mice) may remain significant, but may be only a fraction of that initially estimated (for examples, see 144, 145, 159, 160). The possibility of oligogenic effects must therefore be borne in mind when studying the effect of a single knockout in mice. In addition, the generation of mouse mutants by CRISPR-Cas9 involves targeting the gene in embryonic stem cells. Generally, these stem cells originate from either 129Sv or B6 mice. For stem cells of 129Sv origin, the mutations are often backcrossed into the B6 background to create homozygotes, but the resulting animals have a mixed genetic background (129Sv/B6). The elimination of this mixed background requires further backcrossing onto B6 to eliminate the congenic footprint around the engineering mutation. This is a critical, lengthy, and expensive step, as the mixed background can have a very significant impact on the phenotype of the animals (see Section 12). For example, an LOF mutation of the gene encoding the erythrocyte enzyme biphosphoglycerate mutase (*Bpgm*) protects A/J mice against blood-stage malaria, but has little impact on blood-stage malaria (*P. chabaudi*) in B6 mice (161). However, the same mutation is protective against cerebral malaria (*P. berghei*) in both strains (161). One solution is to introduce the mutation into embryonic stem cells of the same genetic background (B6) subsequently used for crosses to bring the mutation to homozygosity or to test the mutation after its introduction into different genetic backgrounds. Conversely, genetic complementation studies in vivo with different allelic variants (different mutations of the same gene) are easy to perform in mice and may provide additional validation for specific gene effects (152).

Another critical issue in comparisons between humans or mice with infection susceptibility phenotypes (forward genetics) and mice bearing a null allele of a specific gene (reverse genetics) is the type of mouse mutation used to study alterations in the corresponding gene product. Individual mouse mutants are readily available with null or conditional alleles for almost all protein-coding genes, and these are the mutations generally used for studies in mice. Such knockout mice can be used to study recessive complete defects. However, recessive partial deficiencies and disorders that are dominant by haploinsufficiency, negative dominance, or gain of function

(GOF) encountered in humans or discovered by forward genetics approaches in mice are very rarely studied by reverse genetics in mice. Forward genetics studies in mice have revealed the molecular basis of deficiencies underlying dominant disorders (162, 163), and partial or complete deficiencies underlying a recessive trait, and even GOF mutations underlying dominant traits (164, 165). This can be very important, as illustrated by genes for which deletion is lethal in utero, but severely hypomorphic mutations can underlie a key infectious phenotype, as discovered by forward genetics (162). Conversely, conditional knockouts in mice have proved very powerful and constitute a clear additional analytical tool for addressing organ- and cell-specific gene effects contributing to infection phenotypes, aspects that are difficult to address in human studies. Finally, knock-in mutations, in which the exact human allelic variants are reconstructed in mice, can also be very helpful. This approach is seldom used, but its systematic implementation would simplify mouse-human comparisons. Studies of individual mouse knockouts (reverse genetics) are sometimes complicated by the oligogenic nature of the original trait studied, and the fact that the knockout may provide only partial context-dependent information. Moreover, knockout mice, by definition, do not cover the range of genotypes at a given locus, potentially restricting the range and diversity of associated infectious phenotypes. Studies in knockout mice are nevertheless extremely useful, but they could be enhanced by the more systematic analysis of knock-in alleles corresponding to human mutations tested in heterozygous or homozygous animals.

14. IMPROVING MOUSE MODELS THROUGH THE USE OF HUMANIZED MICE

Humanized mice, also known as human immune system (HIS) mice, have been developed to study various functional aspects of human leukocytes in the controlled conditions of an animal model, including the response to infection with real human-specific pathogens. These mice are created by transplanting human $CD34^+$ hematopoietic progenitors from fetal liver, cord blood, or bone marrow into mouse recipients that are immunodeficient due to genetic defects of the development and function of various hematopoietic system components. They were developed for the optimal engraftment and development of human progenitors into mature lymphoid, myeloid, or other lineages, and their use for immunology and oncology is on the increase (for recent reviews see 166, 167). Since the discovery of *nude* mice (*Foxn1* mutation) in the 1960s, and their use as recipients of human tumor xenografts, many new strains with better performance, bearing combinations of different mutations, are now available. These new strains include (*a*) SCID mice (mutation in *Prkdc*), which have a severe defect in T and B cell development and lack cell-mediated and humoral immunity; (*b*) *Rag1* or *Rag2* mutant mice, which are deficient for VDJ recombination; (*c*) nonobese diabetic (NOD) mice (*Sirpa* variant), which allow negative signaling through CD47 in macrophages, improving the tolerance of human cell engraftment; and (*d*) *Il2rg* mutant mice, which display an early impairment of immune cell development, a paucity of mature B and T cells, and an absence of NK cells. The most widely used mutant mouse strains include NOD/SCID/$Il2rg^{-/-}$ (NSG), NOD/*Rag*/ $Il2rg^{-/-}$ (NRG), and BALB/c *Rag2*/ $Il2rg^{-/-}$/$Sirpa^{NOD}$ (BRGS) mice (168). Imbalances in the development of specific lineages (e.g., myeloid) in these mice can be remedied by the administration of human cytokines and growth factors, including human M-CSF (macrophage colony-stimulating factor) and G-CSF (granulocyte colony-stimulating factor).

HIS mice have been reconstituted with $CD34^+$ hematopoietic stem cells (HSCs) and have been used to study various human-specific infectious agents, including viruses, such as HIV, Epstein-Barr virus (EBV), CMV, human adenovirus, filoviruses of the Ebola family, and dengue virus. Several human bacterial pathogens that are only weakly virulent in mice, such as *Salmonella enterica* serovar Typhi, *Mycobacterium tuberculosis* (latency and granuloma formation), and *Staphylococcus aureus* (which has a higher virulence in HIS mice), have also been studied (169).

The human malarial agent *P. falciparum* can also replicate in such mice following the adoptive transfer of mature human erythrocytes, providing a model for testing the efficacy of antimalarial drugs and vaccines (169). HIS mice reconstituted with human HSCs or with HSCs from individual immunodeficient patients therefore provide an attractive complementary model for studying human immunity, including the response to human-specific infections, in a controlled environment. On the other hand, these mice have several limitations reflecting the murine origin of the nonhematopoietic lineages, including bone marrow stromal cells, the dysregulation caused by the coexistence of mouse and human cells, sex effects, the general difficulties procuring HSCs, and allograft rejection for pooled bone marrow donors. One particular concern in studies of immunity to infection is the murine nonhematopoietic component of host defense. Their keratinocytes, pulmonary cells, cardiomyocytes, neurons, and hepatocytes, and most of the hundreds of discernable cell types that can be infected with various viruses, are of murine origin.

15. HUMANS CARRY A GREAT MANY RARE AND COMMON VARIANTS

The genetic variability of the 36 strains of inbred mice studied by whole-genome sequencing is extremely limited relative to the tremendous genetic variability in humans. This genetic variability was long suspected but has only recently been extensively documented, during the last ten years, thanks to the advent of next-generation sequencing (NGS) (170–176). Since 2009, whole-exome sequencing and whole-genome sequencing have made a major contribution in this respect (177, 178). Most exons of protein-coding genes, and their flanking intron regions, together with a large proportion of RNA genes, are sequenced by the latest exome capture kits (179). About a million exomes from subjects from the general population are now available in large public databases, such as gnomAD, which alone provides over 125,000 exomes (**https://gnomad.broadinstitute.org**), and the UK Biobank, which contains 200,000 exomes (**https://www.ukbiobank.ac.uk**). The exomes of a rapidly growing number of patients with well-defined immunological and/or infectious conditions have also been sequenced by investigators worldwide. Fewer whole-genome sequences are available (76,000 in gnomAD), due to the higher costs of generation and storage for such sequences, with only limited benefits in terms of the detection of potentially pathogenic variants. Before NGS, it was difficult to place a mutation identified by candidate-gene or genome-wide approaches in a genomic context, particularly in single-patient studies and, to a lesser extent, single (multiplex) families. These families were known to carry the candidate mutation found, but the rest of the variants remained unknown. This was less of a problem for multiplex families than for sporadic cases, particularly for large kindreds with three or more patients, as genome-wide linkage could, in such instances, identify a single chromosomal region linked with disease. However, even in the region identified, not all variants, and even not all variants in the coding exons of protein-coding genes, were known. These studies were based on forward genetics approaches, but were nevertheless often unable to implicate a single candidate variant nonambiguously. In addition, the prioritization of candidate genes in broad genetic intervals was based largely on available annotations and functional data for the genes present in these intervals, which limited the identification of the morbid gene (if immunologically unannotated). The robustness and ranking of genes within the candidate chromosomal regions selected in forward genetics studies were painstakingly assessed by genome-wide linkage across different kindreds. The availability of multiple kindreds facilitated the discovery of causal genetic lesions. With NGS and the ensuing possibility of placing any variant in the context of the whole exome (or genome) of any patient, the unlimited genetic diversity of humans, which had appeared an unsurmountable obstacle to genetic studies, has become a unique advantage (32, 180, 181). Indeed, it has become possible to attest to the penetrance of the genetic lesion for the trait studied across families and ancestries, and, more specifically, across all other loci. A variant that has the same biological and clinical impact in families and ancestries

sharing no other set of deleterious variants, and with similar penetrance, can be robustly deduced to underlie the infectious phenotype studied. Conversely, the same allele may be pathogenic in the context of specific families or ancestries, pointing to the co-responsibility of other loci.

Variants in the human genome can be classified as rare or common, depending on their minor allele frequency (below or above 1%), in a virtual global population, or in populations corresponding to each of the five major ancestries (African, European, East Asian, South Asian, Latino/admixed American), or even in any specific ethnic or national group. Some LOF alleles that are exceedingly rare in most populations may be common in isolated populations, as exemplified by LOF alleles in IFNAR1 and IFNAR2, which are common in Polynesian and Arctic populations, respectively (181a–181c). Variants may be detected in protein- or RNA-coding genes, and in coding or noncoding exons, or outside exons for protein-coding genes. In the coding regions, the variants can be synonymous or nonsynonymous. The two intronic nucleotides in the vicinity of an exon are typically considered together with nonsynonymous coding variants, as their substitution or deletion is often deleterious. Variants located deeper within the introns may also affect mRNA splicing, if they affect the branch point nucleotides, for example (182). In addition to single nucleotide variants, which include predicted loss of function (pLOF) and missense variants, there are also small insertions and deletions (indels). There is also a great diversity of copy number variants, also known as structural variants (SVs), in the human genome, the deleteriousness of which can be difficult to assess (183). The latest genetic data suggest that there are at least 4 million variants per genome, including about 10,000 nonsynonymous single nucleotide variants, most of which are missense, with a small proportion being nonsense variants. There are also a great many private variants (i.e., found only in single kindreds to date). Indeed, two randomly chosen humans have about 20,000 nucleotide differences between their protein-coding exomes. There are about 100 pLOF variants per genome, only a few of which are homozygous (184). Moreover, there are 166 genes that are homozygous for pLOF alleles in >1% of the human population (52). These variants, with a minor allele frequency (MAF) of >10%, are unlikely to underlie common infectious diseases when present in the homozygous state, whereas they may underlie resistance to infection if driven by positive selection. The mere identification of such a genotype in a patient should not therefore necessarily lead to its automatic implication in the phenotype studied. Causality is not demonstrated simply by the discovery of a pLOF, or even a private variant. The genetic diversity at human loci can be calculated to determine whether these loci have been subjected to negative or positive selection (52, 53, 185). This makes it possible to determine, by inference, whether a given allele, whether LOF or GOF, can plausibly underlie the infectious trait studied. As detailed below, experimental studies ex vivo and in vitro, and mouse studies in vivo, are almost always necessary, in support of in silico studies, to establish causality between a candidate genotype and a clinical phenotype.

16. GENETIC STUDIES OF INFECTIOUS DISEASES IN HUMANS

The almost infinite genetic variability of humans—not to mention their continued somatic genetic diversification during their lifetime, and the epigenetic changes resulting from exposure to an ever-changing environment—has always been a formidable obstacle to the genetic dissection of infection susceptibility in humans (32, 180, 181, 186). Indeed, it is not possible to be 100% certain that a genetic variant is actually responsible for the trait studied unless multiple unrelated patients are studied simultaneously, or segregation or haplotype mapping data are available in a large family. Moreover, the direct immunological study of immunity to infection during the course of disease is rarely fruitful, simply because the immunological abnormalities detected may be the cause of the disease (and possibly, but not necessarily, preexisting infection) or a consequence of or exacerbation due to infection, the disease itself (typically, but not necessarily, absent before

infection), or associated therapeutic interventions. Robust genetics and high-impact immunology results can be generated by studying multiple kindreds with the same infectious phenotype, whether that phenotype is one of resistance or susceptibility. A single gene mutated across affected families and ancestries proves causality beyond any reasonable doubt. This is certainly the case for Mendelian disorders (i.e., with complete penetrance) and also, arguably, for monogenic but not Mendelian disorders (i.e., with incomplete penetrance) (180). Admittedly, the distinction between Mendelian and non-Mendelian monogenic disorders is more operational and didactic than universal and scientific, if only because a monogenic disorder can be Mendelian in patients from a particular ancestry but not another. Monogenic susceptibility to infection appears to be relatively rare, based on current data, but we would argue that this reflects not their actual rarity but an ascertainment bias. Indeed, the human genetic basis of infectious diseases has traditionally been studied from the angle of population-based genetic studies, following on from the landmark discovery of Anthony Allison in 1954 (187–189). Allison found that the sickle cell trait confers a tenfold increase in resistance to severe forms of *P. falciparum* malaria (189). However, over the following ~70 years, the lack of population-based studies yielding results of a similar magnitude has attested to the inherent limitations of this approach. Most of these studies, whether performed by candidate-gene or by genome-wide association approaches, have yielded odds ratios < 2 (186), a notable exception being the study of the spontaneous clearance of hepatitis C virus and the response to type I interferon therapy in chronically infected patients, both of which are strongly associated with common variants at the type III interferon locus (190).

There are several explanations for the limited success of population-based association studies. First, the assumption of phenotypic homogeneity may be invalid (e.g., 2,000 patients tagged as having tuberculosis may not actually have the same condition). Second, the assumption of genetic and allelic homogeneity may also be invalid (e.g., the 2,000 patients may not all have the same genotype conferring predisposition to tuberculosis, whatever its monogenic, digenic, or oligogenic nature). Third, the assumption that common variants (MAF >1%) underlie disease may be invalid (e.g., some of the 2,000 patients may be sick because of common variants, but not all). Fourth, the model tested in most studies is that of dominant or additive inheritance, with recessive inheritance only rarely considered. Fifth, most studies search for genetic effects underlying susceptibility, as opposed to resistance, contrary to Anthony Allison's seminal discovery. Sixth, the necessary statistical correction for multiple error testing may hide signals that are immunologically and causally relevant at the individual if not population level, artificially highlighting statistical significance at the population level at the detriment of immunological causality at the individual level. Seventh, searches for population genetic risk factors, like the sickle cell trait, which are not mechanistically causal of disease in patients, are not designed to reveal truly causal genetic lesions. Instead, they are more likely to reveal the cumulative and historical impact of pathogens on the genetic makeup of the general population rather than the causal mechanism of disease in individual patients. Eighth, the assumption that the study of infectious diseases should be conducted at the population level goes against the notion of each infectious disease resulting from a unique interaction between a singular microbial isolate and a singular human being, individually modulated by complex environmental factors. These aspects relate to the fundamental notions of population thinking, of Ernst Mayr, and chemical individuality, of Archibald Garrod, who followed the concomitant revolutionary insights of Charles Darwin and Claude Bernard that all living organisms are unique in space and time. We would argue that the paucity of known Mendelian or monogenic forms of resistance or susceptibility to infectious diseases results largely from the rarity of studies designed to detect them. Forward genetics studies in mice have convincingly shown that this line of research can be successful; it should be equally successful in humans, who display a much greater diversity of genotypes and phenotypes (191). Moreover, the discovery of monogenic inborn errors

of immunity can operate as a compass, pointing in a mechanistic direction. Other causes, genetic or otherwise, may trigger the same mechanisms of diseases, as illustrated by autoantibodies to cytokines that phenocopy the corresponding inborn errors and can be much more common (2, 32, 82, 181, 192–195).

17. GENETIC STUDIES IN MULTIPLE KINDREDS

There are three classic examples of Mendelian resistance to infection, three autosomal recessive (AR) beneficial deficiencies discovered in multiple kindreds: selective DARC (Duffy antigen receptor for chemokines) deficiency on erythrocytes conferring resistance to *P. vivax*, CCR5 deficiency on T cells conferring resistance to R5 strains of HIV, and FUT2 deficiency on intestinal epithelial cells conferring resistance to norovirus (186). There are more examples in the realm of Mendelian or monogenic predisposition to infectious diseases. We focus here on two infections for which the mouse model has played different roles. The study of multiple kindreds has led to the discovery of rare variants of UNC93B and TLR3 underlying herpes simplex encephalitis (196, 197). This work led to the discovery, occasionally in single patients, of other mutations of the TLR3-dependent pathway governing the induction of type I interferons (198). The causal link between type I interferon deficiency and herpes simplex encephalitis was demonstrated by the study of a single patient with AR IFNAR1 deficiency (199). These findings also led to the establishment of the role of human cortical neurons in the TLR3-dependent cell-intrinsic control of HSV-1 (200, 201), not through TLR3 detecting dsRNA generated in the course of infection, but through TLR3 controlling the baseline, tonic levels of type I interferons in these cells (202). Finally, this study provided novel insights into neuron-intrinsic and brain territory–specific mechanisms of antiviral immunity. By mechanisms that remain to be elucidated but differ from those affected by mutations in the TLR3-IFNAR1 circuit, *SNORA31* mutations underlie forebrain HSE, whereas *DBR1* mutations underlie brainstem HSE (203, 204). HSE is the most common sporadic viral encephalitis in humans. Its sporadic nature is accounted for by the incomplete penetrance of its relatively rare disease-causing genotypes. It is also the first life-threatening, idiopathic, and relatively rare infectious disease of childhood to be shown to be due to, in 5–10% of cases, monogenic lesions. Identification of new causal genes, identification of modifiers of known genes, and elucidation of the pathogenesis of adult HSE are three main goals in the field. The study of HSE by forward and reverse genetics in mice has identified several loci not yet tested in humans (64, 205), whereas studies of the TLR3 pathway in mice have confirmed the findings of human studies (206).

The second example is the study of Mendelian susceptibility to mycobacterial diseases (MSMD), for which the mouse model has proved both seminal and invaluable (207). Over the last 25 years, researchers have identified 15 MSMD-causing genes that, given the additional level of allelic heterogeneity at 7 loci, result in 33 genetic disorders (39) (**Figure 1**). Ironically, most of the MSMD loci were found by studying one of the few experimental infections in humans: vaccination against tuberculosis with live BCG. Most of these disorders were identified either by candidate-gene studies in multiple kindreds before the advent of NGS or by genome-wide approaches after the introduction of NGS. Causal variants with high penetrance are rare or private, as the prevalence of MSMD, which is characterized by selective vulnerability to weakly virulent mycobacteria, is about 1 in 50,000. This high locus and allelic heterogeneity strikingly underlies full immunological homogeneity: All MSMD etiologies disrupt the production of type II interferon (IFN-γ, which acts more as a macrophage-activating factor than as an antiviral interferon) (208) by lymphocytes, or the response of myeloid cells to this molecule. For all the MSMD loci studied in mice, the orthologous knockout mice were highly vulnerable to BCG infection (207). The study of MSMD led to investigations of the monogenic component of susceptibility to tuberculosis. *M. tuberculosis* is about 1,000 times more virulent than the weakly virulent mycobacterial species

responsible for MSMD (infections caused by BCG vaccines and environmental mycobacteria). A genome-wide approach revealed that homozygosity for P1104A in *TYK2* underlies tuberculosis in multiple kindreds, across several ancestries (209). The P1104A allele has a MAF of about 4% in Europeans, is absent from sub-Saharan Africa and very rare in Eastern Asia, and has a MAF of about 1% elsewhere, probably due to genetic admixture. Moreover, this genotype was shown to underlie about 1% of the cases of tuberculosis at whole-population level in the United Kingdom since World War II (210). Finally, it was recently shown that P1104A originated about 8,000 years ago in ancestors of Western Europeans and that its MAF has been decreasing strongly and steadily in Europe over the last 2,000 years, probably attesting to the negative selection imposed by tuberculosis (211). The study of MSMD, thus, led to the discovery of a monogenic and common cause of tuberculosis. These studies were inspired by two independent sets of studies conducted in mice. Landmark studies in reverse genetics showed that *IFNG* knockout mice were highly vulnerable to BCG (212), whereas other forward genetics studies showed that susceptibility to BCG could be attributed to a single locus, *Bcg/Nramp1* (213).

18. GENETIC STUDIES IN SINGLE FAMILIES OR SINGLE PATIENTS

Immunological and genetic studies in single patients have understandably been met with skepticism from the research community. However, a careful analysis of the literature in 2014 revealed that 25% of inborn errors of immunity were first reported in single patients. The initial descriptions have stood the test of time, provided that they meet three stringent criteria (214). First, complete penetrance is required. In other words, there should be no relative carrying the at-risk genotype without the clinical phenotype. This typically limits these studies to recessive traits or de novo dominant mutations. Moreover, the morbid genotype, or its functional equivalents, cannot be found in the general population at a frequency exceeding that of the clinical phenotype of the patient. If there is incomplete penetrance, it must be explained mechanistically, as illustrated for inherited TIRAP (TIR domain–containing adaptor protein) deficiency (215). In this study, only one of the eight relatives with AR TIRAP deficiency had staphylococcal disease. The explanation was provided by incomplete penetrance being due to a lack of antibodies to staphylococcal LTA, an agonist of TIRAP-dependent TLR2, in the patient, whereas his healthy but deficient relatives produced such antibodies, making it possible to bypass the requirement for TIRAP. Second, the variant must be shown to be deleterious, responsible for some degree of loss or gain of function. None of the alleles present in the general population, and especially in the ethnic group of the patient, at an allele frequency greater than the phenotype should display this functional abnormality. Third, a cellular phenotype that serves as an intermediate endophenotype accounting for disease by providing a disease mechanism must be rescued by a wild-type copy of the gene. Alternatively or additionally, a mouse model of the deficiency should reproduce the infectious phenotype. Here again, the mouse model is invaluable for demonstrating the causality between a genotype and a phenotype in a single patient. One example is provided by human *NOS2* deficiency, for which causality for lethal CMV disease in a single patient can be claimed because the corresponding knockout mouse is susceptible to the MCMV (216). With the application of these stringent criteria, the careful and rigorous study of single patients can provide useful insights into the mechanisms of immunity to infection, particularly with support from mouse models.

It should be stressed that studies of single patients have been of unexpected value in the current COVID-19 pandemic. The discovery of AR IRF7 deficiency as the first genetic etiology of critical influenza pneumonitis in a single child (217) led to that of mutations of *TLR3* in three unrelated patients and of *IRF9* in another single patient (218, 219). These studies defined TLR3-dependent, type I and III interferon immunity as essential for host defense against influenza virus in the lungs. By inference, we and the COVID Human Genetic Effort (**https://www.covidhge.com**) tested

the hypothesis that critical COVID-19 is allelic to critical influenza (1). We found mutations at these and related loci, including mutations in two unrelated patients with AR IRF7 deficiency and two other unrelated patients with AR IFNAR1 deficiency, which implicated type I, as opposed to type III, interferon in the pathogenesis of COVID-19 (220). Incidentally, IFNAR1 deficiency had been discovered in two unrelated patients one year earlier (221), whereas IFNAR2 deficiency had been discovered five years earlier in a single patient (222). A genome-wide, unbiased approach also found mutations of *TLR7* underlying 1% of critical cases in male patients under the age of 60 years (223). These rare genotypes, often found in single patients, thus indicated an essential role of type I interferon in human immunity to two unrelated RNA respiratory viruses. Remarkably, they also led to the discovery that at least 20% of critical cases are due to neutralizing autoantibodies against type I interferons (82, 193, 194, 224). Such autoantibodies were not found in any subject with asymptomatic or mild SARS-CoV-2 infection. Their prevalence increases sharply in the general population after the age of 70 years (224). The clinical and immunological similarities between patients with critical disease with and without these autoantibodies (225) suggest that a similar mechanism of disease, albeit due to various causes, is at work in these patients (2, 226). These autoantibodies also account for about a third of adverse reactions to the live attenuated yellow fever vaccine (82) and 5% of cases of critical influenza pneumonia (226a). Overall, in hindsight, the first step toward cracking the enigma of life-threatening COVID-19 pneumonia was the 2015 genetic study of a single child with critical influenza (2, 32). This study, in turn, was based on previous discoveries that type I interferon immunity is crucial for antiviral immunity in mice, including immunity to respiratory viruses, such as influenza virus (227). It also built on the pioneering discovery by forward genetics that mutations of the type I interferon–inducible *Mx1* gene underlie susceptibility to influenza virus in mice (3). A remarkable recent study found that rare variants of the human *MX* gene underlie critical zoonotic avian influenza in China (228). As for tuberculosis, the genetic study of COVID-19 and avian influenza has built on both forward and reverse genetics studies conducted in mice. These examples neatly illustrate the complementarity of solid genetic studies in mice and humans.

19. CONCLUDING REMARKS

We stress here that the immunological mechanisms of resistance or susceptibility to infection are best and most rigorously studied if the germline genetic basis of the individual mouse or human infected is precisely known and carefully analyzed. It is also essential to search for and elucidate causal mechanisms, of a biochemical or immunological nature, that can fully account for the infectious phenotype. The number of mouse strains or individual patients for which infections are studied is not important, provided that a causal relationship and a molecular mechanism are discovered. If the findings are solid, then their genetic basis provides evidence of causality and a mechanism that can guide further endeavors. The host genetics of infectious diseases in laboratory mice or individual patients, with the discovery of monogenic predisposition or resistance, provides a blueprint or roadmap for studies of other mice and humans. Once a causal immunological mechanism is attributed to a particular infectious disease, it becomes much easier to identify possible nonmonogenic causes, genetic or otherwise, likely to disrupt the same immunological mechanism, thereby underlying the same infectious disease (2, 32, 181). The potential genetic causes to be discovered at the individual level include not only etiologies with digenic inheritance but also those with oligogenic inheritance. For a given infection, the level of genetic heterogeneity underlying cases due to these modes of inheritance is probably even greater than that underlying monogenic and even Mendelian cases. This heterogeneity has made a major contribution to the difficulties of population-based approaches. Deciphering the basis of the incomplete penetrance

of monogenic disorders underlying a given infection will probably be the first step toward tackling the genetic architecture of cases with an inheritance that is not strictly Mendelian.

Digenic or oligogenic inheritance may phenocopy the inborn errors of immunity in patients or mice. Somatic mutations may also compromise a proportion of lymphocytes, a particular epithelium, a set of neurons, or a liver lobe, thereby accounting for corresponding infections mimicking those caused by the corresponding inborn errors (229, 230). Alternatively, epigenetic changes in certain cells of the body, including T and B cells, may contribute to the high level of interindividual clinical variability during the course of infection. Finally, autoantibodies may also phenocopy inborn errors, as neatly illustrated by autoantibodies that neutralize cytokines and phenocopy the corresponding inborn errors of the cytokines or their receptors (2, 181, 192, 195, 224). Autoantibodies against IFN-α/β, IFN-γ, IL-6, IL-17A/F, and GM-CSF underlie viral, mycobacterial, staphylococcal, fungal, and nocardial infections, respectively, like the corresponding inborn errors. A germline monogenic basis of infectious diseases may therefore not be found in all patients, and may not even exist in most patients. However, the discovery of such a basis of infection, often driven by prior genetic studies in mice, provides the best starting point for explorations of new, uncharted territories. Meanwhile, when reading a paper reporting a genetic study of mouse or human immunity to infection, it is essential to analyze, with rigor and in depth, whether the genetic foundations of the paper are solid and whether its conclusions respect the principles of genetics. When they do, the similarities between mouse and human studies can be striking. Infectious phenotypes detected in humans are often replicated in mice, the key exceptions typically being due to the lack of an appropriate mouse model of infection. The converse is rarer, even for mouse studies with a solid genetic basis. This is largely because experimental infections in one strain of laboratory mice reveal phenotypes with much greater sensitivity than natural infections in highly diverse human populations. This is actually a good thing, as false negatives in the mouse model would pose an almost insurmountable problem whereas false positives do not prevent in-depth immunological studies of human infections in a mouse model.

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LITERATURE CITED

1. Casanova JL, Su HC, COVID Hum. Genet. Effort. 2020. A global effort to define the human genetics of protective immunity to SARS-CoV-2 infection. *Cell* 181:1194–99
2. Zhang Q, Bastard P, COVID Hum. Genet. Effort, Cobat A, Casanova JL. 2022. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature* 603:587–98
3. Haller O, Arnheiter H, Pavlovic J, Staeheli P. 2018. The discovery of the antiviral resistance gene *Mx*: a story of great ideas, great failures, and some success. *Annu. Rev. Virol.* 5:33–51
4. Staeheli P, Haller O, Boll W, Lindenmann J, Weissmann C. 1986. Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. *Cell* 44:147–58
5. Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, et al. 1987. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. *Nature* 330:576–78
6. Thomas KR, Capecchi MR. 1987. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 51:503–12
7. Quintana-Murci L, Alcaïs A, Abel L, Casanova JL. 2007. Immunology in natura: clinical, epidemiological and evolutionary genetics of infectious diseases. *Nat. Immunol.* 8:1165–71
8. Pulendran B, Davis MM. 2020. The science and medicine of human immunology. *Science* 369:eaay4014
9. Hayday AC, Peakman M. 2008. The habitual, diverse and surmountable obstacles to human immunology research. *Nat. Immunol.* 9:575–80
10. Davis MM, Brodin P. 2018. Rebooting human immunology. *Annu. Rev. Immunol.* 36:843–64
11. Davis MM. 2008. A prescription for human immunology. *Immunity* 29:835–38
12. Casanova JL, Abel L. 2004. The human model: a genetic dissection of immunity to infection in natural conditions. *Nat. Rev. Immunol.* 4:55–66
13. White R, Caskey CT. 1988. The human as an experimental system in molecular genetics. *Science* 240(4858):1483–88
14. Medetgul-Ernar K, Davis MM. 2022. Standing on the shoulders of mice. *Immunity* 55(8):1343–53
15. Casanova JL, Abel L. 2002. Genetic dissection of immunity to mycobacteria: the human model. *Annu. Rev. Immunol.* 20:581–620
16. Casanova J-L, Nathan CF, Nussenzweig MC. 2016. Human studies at *JEM*: immunology and beyond. *J. Exp. Med.* 213:467–68
17. Bonthron DT, Markham AF, Ginsburg D, Orkin SH. 1985. Identification of a point mutation in the adenosine deaminase gene responsible for immunodeficiency. *J. Clin. Investig.* 76:894–97
18. Lutz W. 1946. A propos de l'epidermodysplasie verruciforme. *Dermatologica* 92:30–43
19. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, et al. 2020. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. *J. Clin. Immunol.* 40:24–64. Erratum. 2020. *J. Clin. Immunol.* 40:65
20. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, et al. 2021. The ever-increasing array of novel inborn errors of immunity: an interim update by the IUIS committee. *J. Clin. Immunol.* 41:666–79
21. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, et al. 2020. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. *J. Clin. Immunol.* 40:66–81
22. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, et al. 2016. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532:512–16
23. Masopust D, Sivula CP, Jameson SC. 2017. Of mice, dirty mice, and men: using mice to understand human immunology. *J. Immunol.* 199:383–88

24. Mestas J, Hughes CC. 2004. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172:2731–38
25. Sellers RS. 2017. Translating mouse models. *Toxicol. Pathol.* 45:134–45
26. Tao L, Reese TA. 2017. Making mouse models that reflect human immune responses. *Trends Immunol.* 38:181–93
27. Paludan SR, Mogensen TH, Pradeu T, Masters SL. 2020. Constitutive immune mechanisms: mediators of host defence and immune regulation. *Nat. Rev. Immunol.* 21:137–50
28. Randow F, MacMicking JD, James LC. 2013. Cellular self-defense: how cell-autonomous immunity protects against pathogens. *Science* 340:701–6
29. Zhang SY, Jouanguy E, Zhang Q, Abel L, Puel A, Casanova JL. 2019. Human inborn errors of immunity to infection affecting cells other than leukocytes: from the immune system to the whole organism. *Curr. Opin. Immunol.* 59:88–100
30. Gaudet RG, Zhu S, Halder A, Kim BH, Bradfield CJ, et al. 2021. A human apolipoprotein L with detergent-like activity kills intracellular pathogens. *Science* 373:eabf8113
31. Nathan C. 2021. Rethinking immunology. *Science* 373:276–77
32. Casanova JL, Abel L. 2021. Mechanisms of viral inflammation and disease in humans. *Science* 374(6571):1080–86
33. Crowther MD, Sewell AK. 2021. The burgeoning role of MR1-restricted T-cells in infection, cancer and autoimmune disease. *Curr. Opin. Immunol.* 69:10–17
34. Papalexi E, Satija R. 2018. Single-cell RNA sequencing to explore immune cell heterogeneity. *Nat. Rev. Immunol.* 18:35–45
35. de Jong SJ, Créquer A, Matos I, Hum D, Gunasekharan V, et al. 2018. The human CIB1-EVER1-EVER2 complex governs keratinocyte-intrinsic immunity to β-papillomaviruses. *J. Exp. Med.* 215:2289–310
36. Spurgeon ME, Lambert PF. 2020. *Mus musculus* papillomavirus 1: a new frontier in animal models of papillomavirus pathogenesis. *J. Virol.* 94:e00002-20
37. von Bernuth H, Picard C, Puel A, Casanova JL. 2012. Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. *Eur. J. Immunol.* 42:3126–35
38. Casanova J-L, Abel L, Quintana-Murci L. 2011. Human TLRs and IL-1Rs in host defense: natural insights from evolutionary, epidemiological, and clinical genetics. *Annu. Rev. Immunol.* 29:447–91
39. Yang R, Mele F, Worley L, Langlais D, Rosain J, et al. 2020. Human T-bet governs innate and innate-like adaptive IFN-γ immunity against mycobacteria. *Cell* 183:1826–47
40. Pöyhönen L, Bustamante J, Casanova J-L, Jouanguy E, Zhang Q. 2019. Life-threatening infections due to live-attenuated vaccines: early manifestations of inborn errors of immunity. *J. Clin. Immunol.* 39:376–90
41. Yeung F, Chen Y-H, Lin J-D, Leung JM, McCauley C, et al. 2020. Altered immunity of laboratory mice in the natural environment is associated with fungal colonization. *Cell Host Microbe* 27:809–22
42. Ramanan D, Tang MS, Bowcutt R, Loke P, Cadwell K. 2014. Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal *Bacteroides vulgatus*. *Immunity* 41:311–24
43. Keubler LM, Buettner M, Häger C, Bleich A. 2015. A multihit model: colitis lessons from the interleukin-10-deficient mouse. *Inflamm. Bowel Dis.* 21:1967–75
44. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, et al. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139:485–98
45. Iliev ID, Cadwell K. 2021. Effects of intestinal fungi and viruses on immune responses and inflammatory bowel diseases. *Gastroenterology* 160:1050–66
46. Cadwell K, Patel KK, Maloney NS, Liu T-C, Ng ACY, et al. 2010. Virus-plus-susceptibility gene interaction determines Crohn's disease gene *Atg16L1* phenotypes in intestine. *Cell* 141:1135–45
47. Boehm T, Hirano M, Holland SJ, Das S, Schorpp M, Cooper MD. 2018. Evolution of alternative adaptive immune systems in vertebrates. *Annu. Rev. Immunol.* 36:19–42
48. Casanova JL, Abel L. 2015. Disentangling inborn and acquired immunity in human twins. *Cell* 160:13–15
49. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, et al. 2015. Variation in the human immune system is largely driven by non-heritable influences. *Cell* 160:37–47

50. Puelma Touzel M, Walczak AM, Mora T, Davenport MP. 2020. Inferring the immune response from repertoire sequencing. *PLOS Comput. Biol.* 16:e1007873
51. Davenport MP, Smith NL, Rudd BD. 2020. Building a T cell compartment: how immune cell development shapes function. *Nat. Rev. Immunol.* 20:499–506
52. Rausell A, Luo Y, Lopez M, Seeleuthner Y, Rapaport F, et al. 2020. Common homozygosity for predicted loss-of-function variants reveals both redundant and advantageous effects of dispensable human genes. *PNAS* 117:13626–36
53. Quintana-Murci L. 2019. Human immunology through the lens of evolutionary genetics. *Cell* 177:184–99
54. Laval G, Peyrégne S, Zidane N, Harmant C, Renaud F, et al. 2019. Recent adaptive acquisition by African rainforest hunter-gatherers of the late Pleistocene sickle-cell mutation suggests past differences in malaria exposure. *Am. J. Hum. Genet.* 104:553–61
55. Kanjee U, Grüring C, Babar P, Meyers A, Dash R, et al. 2021. *Plasmodium vivax* strains use alternative pathways for invasion. *J. Infect. Dis.* 223(10):1817–21
56. Malone KM, Gordon SV. 2017. *Mycobacterium tuberculosis* complex members adapted to wild and domestic animals. *Adv. Exp. Med. Biol.* 1019:135–54
57. Klingel K, Stephan S, Sauter M, Zell R, McManus BM, et al. 1996. Pathogenesis of murine enterovirus myocarditis: virus dissemination and immune cell targets. *J. Virol.* 70:8888–95
58. Feuer R, Mena I, Pagarigan RR, Harkins S, Hassett DE, Whitton JL. 2003. Coxsackievirus B3 and the neonatal CNS: the roles of stem cells, developing neurons, and apoptosis in infection, viral dissemination, and disease. *Am. J. Pathol.* 163:1379–93
59. Somova LM, Kondrashova NM, Plekhova NG, Drobot EI, Lyapun IN. 2013. Pathomorphosis of experimental infection in mice, infected by *Streptococcus pneumoniae*, under the effect of immunotropic drugs. *Bull. Exp. Biol. Med.* 155:477–83
60. Mrochen DM, Fernandes de Oliveira LM, Raafat D, Holtfreter S. 2020. *Staphylococcus aureus* host tropism and its implications for murine infection models. *Int. J. Mol. Sci.* 21:7061
61. Kim HK, Missiakas D, Schneewind O. 2014. Mouse models for infectious diseases caused by *Staphylococcus aureus*. *J. Immunol. Methods* 410:88–99
62. Radovanovic I, Mullick A, Gros P. 2011. Genetic control of susceptibility to infection with *Candida albicans* in mice. *PLOS ONE* 6:e18957
63. Gaffen SL, Moutsopoulos NM. 2020. Regulation of host-microbe interactions at oral mucosal barriers by type 17 immunity. *Sci. Immunol.* 5:eaau4594
64. Hayes CK, Wilcox DR, Yang Y, Coleman GK, Brown MA, Longnecker R. 2021. ASC-dependent inflammasomes contribute to immunopathology and mortality in herpes simplex encephalitis. *PLOS Pathog.* 17:e1009285
65. Montes de Oca M, de Labastida Rivera F, Winterford C, Frame TCM, Ng SS, et al. 2020. IL-27 signalling regulates glycolysis in Th1 cells to limit immunopathology during infection. *PLOS Pathog.* 16:e1008994
66. Chen M, Sun H, Boot M, Shao L, Chang SJ, et al. 2020. Itaconate is an effector of a Rab GTPase cell-autonomous host defense pathway against *Salmonella*. *Science* 369:450–55
67. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, et al. 2018. Influenza. *Nat. Rev. Dis. Primers* 4(1):3
68. Graham AL. 2021. Naturalizing mouse models for immunology. *Nat. Immunol.* 22:111–17
69. Fischer AW, Cannon B, Nedergaard J. 2018. Optimal housing temperatures for mice to mimic the thermal environment of humans: an experimental study. *Mol. Metab.* 7:161–70
70. Hopwood TW, Hall S, Begley N, Forman R, Brown S, et al. 2018. The circadian regulator BMAL1 programmes responses to parasitic worm infection via a dendritic cell clock. *Sci. Rep.* 8:3782
71. Druzd D, Matveeva O, Ince L, Harrison U, He W, et al. 2017. Lymphocyte circadian clocks control lymph node trafficking and adaptive immune responses. *Immunity* 46:120–32
72. Knudsen NH, Stanya KJ, Hyde AL, Chalom MM, Alexander RK, et al. 2020. Interleukin-13 drives metabolic conditioning of muscle to endurance exercise. *Science* 368:eaat3987
73. Witjes VM, Boleij A, Halffman W. 2020. Reducing versus embracing variation as strategies for reproducibility: the microbiome of laboratory mice. *Animals* 10(12):2415

74. Jeyakumar T, Beauchemin N, Gros P. 2019. Impact of the microbiome on the human genome. *Trends Parasitol*. 35:809–21
75. MacDuff DA, Reese TA, Kimmey JM, Weiss LA, Song C, et al. 2015. Phenotypic complementation of genetic immunodeficiency by chronic herpesvirus infection. *eLife* 4:e04494
76. Fallon MT, Benjamin WH, Schoeb TR, Briles DE. 1991. Mouse hepatitis virus strain UAB infection enhances resistance to *Salmonella typhimurium* in mice by inducing suppression of bacterial growth. *Infect. Immun.* 59:852–56
77. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, et al. 2007. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447:326–29
78. Hamilton SE, Badovinac VP, Beura LK, Pierson M, Jameson SC, et al. 2020. New insights into the immune system using dirty mice. *J. Immunol.* 205:3–11
79. Lin J-D, Devlin JC, Yeung F, McCauley C, Leung JM, et al. 2020. Rewilding Nod2 and Atg16l1 mutant mice uncovers genetic and environmental contributions to microbial responses and immune cell composition. *Cell Host Microbe* 27:830–40
80. Casanova JL, Zhang Q, Bastard P, Jouanguy E. 2022. In memoriam: Stephen J Seligman, MD. *J. Clin. Immunol.* 42:437–40
81. Gothe F, Howarth S, Duncan CJ, Hambleton S. 2021. Monogenic susceptibility to live viral vaccines. *Curr. Opin. Immunol.* 72:167–75
82. Bastard P, Michailidis E, Hoffmann HH, Chbihi M, Le Voyer T, et al. 2021. Auto-antibodies to type I IFNs can underlie adverse reactions to yellow fever live attenuated vaccine. *J. Exp. Med.* 218:e20202486
83. Zhang YE, Vibranovski MD, Landback P, Marais GA, Long M. 2010. Chromosomal redistribution of male-biased genes in mammalian evolution with two bursts of gene gain on the X chromosome. *PLOS Biol.* 8:e1000494
84. Nei M, Xu P, Glazko G. 2001. Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. *PNAS* 98:2497–502
85. White TD, Asfaw B, Beyene Y, Haile-Selassie Y, Lovejoy CO, et al. 2009. *Ardipithecus ramidus* and the paleobiology of early hominids. *Science* 326:64–86
86. Love GD, Grosjean E, Stalvies C, Fike DA, Grotzinger JP, et al. 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature* 457:718–21
87. Smith JJ, Kuraku S, Holt C, Sauka-Spengler T, Jiang N, et al. 2013. Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. *Nat. Genet.* 45:415–21
88. Anderson PS, Friedman M, Brazeau MD, Rayfield EJ. 2011. Initial radiation of jaws demonstrated stability despite faunal and environmental change. *Nature* 476:206–9
89. Churakov G, Sadasivuni MK, Rosenbloom KR, Huchon D, Brosius J, Schmitz J. 2010. Rodent evolution: back to the root. *Mol. Biol. Evol.* 27:1315–26
90. Boursot P, Auffray JC, Britton-Davidian J, Bonhomme F. 1993. The evolution of house mice. *Ann. Rev. Ecol. Syst.* 24:119–52
91. Kolishovski G, Lamoureux A, Hale P, Richardson JE, Recla JM, et al. 2019. The JAX Synteny Browser for mouse-human comparative genomics. *Mamm. Genome* 30:353–61
92. Emes RD, Goodstadt L, Winter EE, Ponting CP. 2003. Comparison of the genomes of human and mouse lays the foundation of genome zoology. *Hum. Mol. Genet.* 12:701–9
93. Zhang X, Firestein S. 2002. The olfactory receptor gene superfamily of the mouse. *Nat. Neurosci.* 5:124–33
94. Young JM, Friedman C, Williams EM, Ross JA, Tonnes-Priddy L, Trask BJ. 2002. Different evolutionary processes shaped the mouse and human olfactory receptor gene families. *Hum. Mol. Genet.* 11:535–46
95. Westgaard IH, Berg SF, Ørstavik S, Fossum S, Dissen E. 1998. Identification of a human member of the Ly-49 multigene family. *Eur. J. Immunol.* 28:1839–46
96. Higuchi DA, Cahan P, Gao J, Ferris ST, Poursine-Laurent J, et al. 2010. Structural variation of the mouse natural killer gene complex. *Genes Immun.* 11(8):637–48
97. Yawata M, Yawata N, Abi-Rached L, Parham P. 2002. Variation within the human killer cell immunoglobulin-like receptor (KIR) gene family. *Crit. Rev. Immunol.* 22(5–6):463–82
98. Webb JR, Lee SH, Vidal SM. 2002. Genetic control of innate immune responses against cytomegalovirus: MCMV meets its match. *Genes Immun.* 3(5):250–62

99. Growney JD, Dietrich WF. 2000. High-resolution genetic and physical map of the Lgn1 interval in C57BL/6J implicates Naip2 or Naip5 in *Legionella pneumophila* pathogenesis. *Genome Res.* 10:1158–71
100. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, et al. 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420:520–62
101. Yeager M, Hughes AL. 1999. Evolution of the mammalian MHC: natural selection, recombination, and convergent evolution. *Immunol. Rev.* 167:45–58
102. Mashimo T, Simon-Chazottes D, Guénet JL. 2008. Innate resistance to flavivirus infections and the functions of 2′-5′ oligoadenylate synthetases. *Curr. Top. Microbiol. Immunol.* 321:85–100
103. Cotton RN, Shahine A, Rossjohn J, Moody DB. 2018. Lipids hide or step aside for CD1-autoreactive T cell receptors. *Curr. Opin. Immunol.* 52:93–99
104. Hardy MP, Owczarek CM, Jermiin LS, Ejdebäck M, Hertzog PJ. 2004. Characterization of the type I interferon locus and identification of novel genes. *Genomics* 84:331–45
105. Oritani K, Kincade PW, Zhang C, Tomiyama Y, Matsuzawa Y. 2001. Type I interferons and limitin: a comparison of structures, receptors, and functions. *Cytokine Growth Factor Rev.* 12:337–48
106. Oritani K, Medina KL, Tomiyama Y, Ishikawa J, Okajima Y, et al. 2000. Limitin: an interferon-like cytokine that preferentially influences B-lymphocyte precursors. *Nat. Med.* 6:659–66
107. Xu L, Yang L, Liu W. 2013. Distinct evolution process among type I interferon in mammals. *Protein Cell* 4(5):383–92
108. Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, et al. 2008. Lymphoproliferative disease and autoimmunity in mice with increased miR-17–92 expression in lymphocytes. *Nat. Immunol.* 9:405–14
109. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, et al. 2008. Targeted deletion reveals essential and overlapping functions of the miR-17 ∼92 family of miRNA clusters. *Cell* 132:875–86
110. Chandan K, Gupta M, Sarwat M. 2020. Role of host and pathogen-derived microRNAs in immune regulation during infectious and inflammatory diseases. *Front. Immunol.* 10:3081
111. Le Pendu J, Ruvoën-Clouet N. 2020. Fondness for sugars of enteric viruses confronts them with human glycans genetic diversity. *Hum. Genet.* 139:903–10
112. Kaur H, Sehgal R, Rani S. 2019. Duffy antigen receptor for chemokines (DARC) and susceptibility to *Plasmodium vivax* malaria. *Parasitol. Int.* 71:73–75
113. Torre S, Langlais D, Gros P. 2018. Genetic analysis of cerebral malaria in the mouse model infected with *Plasmodium berghei*. *Mamm. Genome* 29:488–506
114. Min-Oo G, Gros P. 2005. Erythrocyte variants and the nature of their malaria protective effect. *Cell. Microbiol.* 7:753–63
115. López C, Saravia C, Gomez A, Hoebeke J, Patarroyo MA. 2010. Mechanisms of genetically-based resistance to malaria. *Gene* 467:1–12
116. Phifer-Rixey M, Nachman MW. 2015. Insights into mammalian biology from the wild house mouse *Mus musculus*. *eLife* 4:e05959
117. Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, et al. 2007. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature* 448:1050–53
118. Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, et al. 2000. Genealogies of mouse inbred strains. *Nat. Genet.* 24:23–25
119. Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, et al. 2011. Non-canonical inflammasome activation targets caspase-11. *Nature* 479:117–21
120. Broz P, Ruby T, Belhocine K, Bouley DM, Kayagaki N, et al. 2012. Caspase-11 increases susceptibility to *Salmonella* infection in the absence of caspase-1. *Nature* 490:288–91
121. Malo D, Vogan K, Vidal S, Hu J, Cellier M, et al. 1994. Haplotype mapping and sequence analysis of the mouse *Nramp* gene predict susceptibility to infection with intracellular parasites. *Genomics* 23:51–61
122. Patel SN, Berghout J, Lovegrove FE, Ayi K, Conroy A, et al. 2008. C5 deficiency and C5a or C5aR blockade protects against cerebral malaria. *J. Exp. Med.* 205:1133–43
123. Radovanovic I, Leung V, Iliescu A, Bongfen SE, Mullick A, et al. 2014. Genetic control of susceptibility to *Candida albicans* in SM/J mice. *J. Immunol.* 193:1290–300
124. Fortin A, Diez E, Rochefort D, Laroche L, Malo D, et al. 2001. Recombinant congenic strains derived from A/J and C57BL/6J: a tool for genetic dissection of complex traits. *Genomics* 74:21–35

125. Fortin A, Belouchi A, Tam MF, Cardon L, Skamene E, et al. 1997. Genetic control of blood parasitaemia in mouse malaria maps to chromosome 8. *Nat. Genet.* 17:382–83
126. Foote SJ, Burt RA, Baldwin TM, Presente A, Roberts AW, et al. 1997. Mouse loci for malaria-induced mortality and the control of parasitaemia. *Nat. Genet.* 17:380–81
127. Ciancanelli MJ, Abel L, Zhang SY, Casanova JL. 2016. Host genetics of severe influenza: from mouse Mx1 to human IRF7. *Curr. Opin. Immunol.* 38:109–20
128. Torre S, van Bruggen R, Kennedy JM, Berghout J, Bongfen SE, et al. 2013. Susceptibility to lethal cerebral malaria is regulated by epistatic interaction between chromosome 4 (*Berr6*) and chromosome 1 (*Berr7*) loci in mice. *Genes Immun.* 14:249–57
129. Poltorak A, He X, Smirnova I, Liu M-Y, Van Huffel C, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 282:2085–88
130. Thomas JD, Smith CIE, Sideras P, Vořechovský I, Chapman V, Paul WE. 1993. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* 261:355–58
131. Rawlings DJ, Saffran DC, Tsukada S, Largaespada DA. 1993. Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science* 261:358–61
132. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, et al. 2001. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27:68–73
133. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, et al. 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27:18–20
134. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, et al. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27:20–21
135. Mahajan VS, Demissie E, Mattoo H, Viswanadham V, Varki A, et al. 2016. Striking immune phenotypes in gene-targeted mice are driven by a copy-number variant originating from a commercially available C57BL/6 strain. *Cell Rep.* 15:1901–9
136. Turcotte K, Gauthier S, Mitsos LM, Shustik C, Copeland NG, et al. 2004. Genetic control of myeloproliferation in BXH-2 mice. *Blood* 103:2343–50
137. Turcotte K, Gauthier S, Tuite A, Mullick A, Malo D, Gros P. 2005. A mutation in the *Icsbp1* gene causes susceptibility to infection and a chronic myeloid leukemia-like syndrome in BXH-2 mice. *J. Exp. Med.* 201:881–90
138. Tailor P, Tamura T, Morse HC 3rd, Ozato K. 2008. The BXH2 mutation in IRF8 differentially impairs dendritic cell subset development in the mouse. *Blood* 111:1942–45
139. Salem S, Salem D, Gros P. 2020. Role of IRF8 in immune cells functions, protection against infections, and susceptibility to inflammatory diseases. *Hum. Genet.* 139:707–21
140. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, et al. 2011. IRF8 mutations and human dendritic-cell immunodeficiency. *N. Engl. J. Med.* 365:127–38
141. Brown MG, Dokun AO, Heusel JW, Smith HR, Beckman DL, et al. 2001. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* 292(5518):934–37
142. Min-Oo G, Fortin A, Tam MF, Nantel A, Stevenson MM, Gros P. 2003. Pyruvate kinase deficiency in mice protects against malaria. *Nat. Genet.* 35:357–62
143. Fortin A, Cardon LR, Tam M, Skamene E, Stevenson MM, Gros P. 2001. Identification of a new malaria susceptibility locus (Char4) in recombinant congenic strains of mice. *PNAS* 98:10793–8
144. Min-Oo G, Fortin A, Pitari G, Tam M, Stevenson MM, Gros P. 2007. Complex genetic control of susceptibility to malaria: positional cloning of the *Char9* locus. *J. Exp. Med.* 204:511–24
145. Laroque A, Min-Oo G, Tam M, Ponka P, Stevenson MM, Gros P. 2017. The mouse *Char10* locus regulates severity of pyruvate kinase deficiency and susceptibility to malaria. *PLOS ONE* 12:e0177818
146. Huang HM, McMorran BJ, Foote SJ, Burgio G. 2018. Host genetics in malaria: lessons from mouse studies. *Mamm. Genome* 29:507–22
147. Torre S, Faucher SP, Fodil N, Bongfen SE, Berghout J, et al. 2015. THEMIS is required for pathogenesis of cerebral malaria and protection against pulmonary tuberculosis. *Infect. Immun.* 83:759–68

148. Kennedy JM, Georges A, Bassenden AV, Vidal SM, Berghuis AM, et al. 2020. ZBTB7B (ThPOK) is required for pathogenesis of cerebral malaria and protection against pulmonary tuberculosis. *Infect. Immun.* 88:e00845-19
149. Bongfen SE, Rodrigue-Gervais IG, Berghout J, Torre S, Cingolani P, et al. 2012. An N-ethyl-N-nitrosourea (ENU)-induced dominant negative mutation in the JAK3 kinase protects against cerebral malaria. *PLOS ONE* 7:e31012
150. Olivier JF, Fodil N, Al Habyan S, Gopal A, Artusa P, et al. 2020. CCDC88B is required for mobility and inflammatory functions of dendritic cells. *J. Leukoc. Biol.* 108:1787–802
151. Kennedy JM, Fodil N, Torre S, Bongfen SE, Olivier JF, et al. 2014. CCDC88B is a novel regulator of maturation and effector functions of T cells during pathological inflammation. *J. Exp. Med.* 211:2519–35
152. Torre S, Polyak MJ, Langlais D, Fodil N, Kennedy JM, et al. 2017. USP15 regulates type I interferon response and is required for pathogenesis of neuroinflammation. *Nat. Immunol.* 18:54–63
153. Hoebe K, Beutler B. 2008. Forward genetic analysis of TLR-signaling pathways: an evaluation. *Adv. Drug. Deliv. Rev.* 60:824–29
154. Crozat K, Georgel P, Rutschmann S, Mann N, Du X, et al. 2006. Analysis of the MCMV resistome by ENU mutagenesis. *Mamm. Genome* 17:398–406
155. Wang T, Bu CH, Hildebrand S, Jia G, Siggs OM, et al. 2018. Probability of phenotypically detectable protein damage by ENU-induced mutations in the Mutagenetix database. *Nat. Commun.* 9:441
156. Arnold CN, Barnes MJ, Berger M, Blasius AL, Brandl K, et al. 2012. ENU-induced phenovariance in mice: inferences from 587 mutations. *BMC Res. Notes* 5:577
157. Papathanasiou P, Goodnow CC. 2005. Connecting mammalian genome with phenome by ENU mouse mutagenesis: gene combinations specifying the immune system. *Annu. Rev. Genet.* 39:241–62
158. Mitsos LM, Cardon LR, Ryan L, LaCourse R, North RJ, Gros P. 2003. Susceptibility to tuberculosis: a locus on mouse chromosome 19 (*Trl-4*) regulates *Mycobacterium tuberculosis* replication in the lungs. *PNAS* 100:6610–15
159. Marquis J-F, LaCourse R, Ryan L, North RJ, Gros P. 2009. Genetic and functional characterization of the mouse *Trl3* locus in defense against tuberculosis. *J. Immunol.* 182:3757–67
160. Potter M, O'Brien AD, Skamene E, Gros P, Forget A, et al. 1983. A BALB/c congenic strain of mice that carries a genetic locus (*Ity*^{r}) controlling resistance to intracellular parasites. *Infect. Immun.* 40:1234–35
161. Xu G, van Bruggen R, Gualtieri CO, Moradin N, Fois A, et al. 2020. Bisphosphoglycerate mutase deficiency protects against cerebral malaria and severe malaria-induced anemia. *Cell Rep.* 32:108170
162. Kibar Z, Vogan KJ, Groulx N, Justice MJ, Underhill DA, Gros P. 2001. *Ltap*, a mammalian homolog of *Drosophila Strabismus/Van Gogh*, is altered in the mouse neural tube mutant Loop-tail. *Nat. Genet.* 28:251–55
163. Epstein DJ, Vekemans M, Gros P. 1991. *splotch* (*Sp2H*), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of *Pax-3*. *Cell* 67:767–74
164. Lewis G, Rapsomaniki E, Bouriez T, Crockford T, Ferry H, et al. 2004. Hyper IgE in New Zealand black mice due to a dominant-negative CD23 mutation. *Immunogenetics* 56:564–71
165. Meng G, Zhang F, Fuss I, Kitani A, Strober W. 2009. A mutation in the *Nlrp3* gene causing inflammasome hyperactivation potentiates Th17 cell-dominant immune responses. *Immunity* 30:860–74
166. Stripecke R, Münz C, Schuringa JJ, Bissig K-D, Soper B, et al. 2020. Innovations, challenges, and minimal information for standardization of humanized mice. *EMBO Mol. Med.* 12:e8662
167. Li Y, Di Santo JP. 2019. Modeling infectious diseases in mice with a "humanized" immune system. *Microbiol. Spectr.* 7. **https://doi.org/10.1128/microbiolspec.BAI-0019-2019**
168. Allen TM, Brehm MA, Bridges S, Ferguson S, Kumar P, et al. 2019. Humanized immune system mouse models: progress, challenges and opportunities. *Nat. Immunol.* 20:770–74
169. Chen Q, Amaladoss A, Ye W, Liu M, Dummler S, et al. 2014. Human natural killer cells control *Plasmodium falciparum* infection by eliminating infected red blood cells. *PNAS* 111:1479–84
170. Sudmant PH, Rausch T, Gardner EJ, Handsaker RE, Abyzov A, et al. 2015. An integrated map of structural variation in 2,504 human genomes. *Nature* 526:75–81
171. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, et al. 2021. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581:434–43

172. Collins RL, Brand H, Karczewski KJ, Zhao X, Alföldi J, et al. 2020. A structural variation reference for medical and population genetics. *Nature* 581:444–51. Erratum. 2021. *Nature* 590:E55
173. Berberich AJ, Ho R, Hegele RA. 2018. Whole genome sequencing in the clinic: empowerment or too much information? *CMAJ* 190:E124–25
174. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. 2015. A global reference for human genetic variation. *Nature* 526:68–74
175. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* 491:56–65
176. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, et al. 2010. A map of human genome variation from population-scale sequencing. *Nature* 467:1061–73
177. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, et al. 2009. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *PNAS* 106:19096–101
178. Shendure J, Waterston RH, Balasubramanian S, Church GM, Gilbert W, et al. 2019. DNA sequencing at 40: past, present and future. *Nature* 550:345–53. Erratum. 2019. *Nature* 568:E11
179. Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, et al. 2015. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. *PNAS* 112:5473–78
180. Casanova J-L, Abel L. 2021. Lethal infectious diseases as inborn errors of immunity: toward a synthesis of the germ and genetic theories. *Annu. Rev. Pathol. Mech. Dis.* 16:23–50
181. Casanova JL, Abel L. 2022. From rare disorders of immunity to common determinants of infection: following the mechanistic thread. *Cell* 185(17):3086–103
181a. Meyts I. 2022. Null IFNAR1 and IFNAR2 alleles are surprisingly common in the Pacific and Arctic. *J. Exp. Med.* 219(6):e20220491
181b. Duncan CJA, Skouboe MK, Howarth S, Hollensen AK, Chen R, et al. 2022. Life-threatening viral disease in a novel form of autosomal recessive IFNAR2 deficiency in the Arctic. *J. Exp. Med.* 219(6):e20212427
181c. Bastard P, Hsiao KC, Zhang Q, Choin J, Best E, et al. 2022. A loss-of-function IFNAR1 allele in Polynesia underlies severe viral diseases in homozygotes. *J. Exp. Med.* 219(6):e20220028
182. Knapp KM, Sullivan R, Murray J, Gimenez G, Arn P, et al. 2020. Linked-read genome sequencing identifies biallelic pathogenic variants in *DONSON* as a novel cause of Meier-Gorlin syndrome. *J. Med. Genet.* 57(3):195–202
183. Yusuff T, Kellaris G, Girirajan S, Katsanis N. 2021. Dissecting the complexity of CNV pathogenicity: insights from *Drosophila* and zebrafish models. *Curr. Opin. Genet. Dev.* 68:79–87
184. MacArthur DG, Balasubramanian S, Frankish A, Huang N, Morris J, et al. 2012. A systematic survey of loss-of-function variants in human protein-coding genes. *Science* 335:823–28
185. Rapaport F, Boisson B, Gregor A, Béziat V, Boisson-Dupuis S, et al. 2021. Negative selection on human genes underlying inborn errors depends on disease outcome and both the mode and mechanism of inheritance. *PNAS* 118:e2001248118
186. Casanova J-L, Abel L. 2020. The human genetic determinism of life-threatening infectious diseases: genetic heterogeneity and physiological homogeneity? *Hum. Genet.* 139:681–94
187. Casanova JL. 2015. Human genetic basis of interindividual variability in the course of infection. *PNAS* 112:7118–27
188. Casanova JL. 2015. Severe infectious diseases of childhood as monogenic inborn errors of immunity. *PNAS* 112:7128–37
189. Allison AC. 1954. Protection afforded by sickle-cell trait against subtertian malarial infection. *Br. Med. J.* 1:290–94
190. Schwerk J, Negash A, Savan R, Gale M Jr. 2021. Innate immunity in hepatitis C virus infection. *Cold Spring Harb. Perspect. Med.* 11:a036988
191. Vidal SM, Malo D, Marquis JF, Gros P. 2008. Forward genetic dissection of immunity to infection in the mouse. *Annu. Rev. Immunol.* 26:81–132
192. Puel A, Bastard P, Bustamante J, Casanova JL. 2022. Human autoantibodies underlying infectious diseases. *J. Exp. Med.* 219(4):e20211387
193. Bastard P, Orlova E, Sozaeva L, Lévy R, James A, et al. 2021. Preexisting autoantibodies to type I IFNs underlie critical COVID-19 pneumonia in patients with APS-1. *J. Exp. Med.* 218:e20210554

194. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann H-H, et al. 2020. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 370:eabd4585
195. Ku CL, Chi CY, von Bernuth H, Doffinger R. 2020. Autoantibodies against cytokines: phenocopies of primary immunodeficiencies? *Hum. Genet.* 139:783–94
196. Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, et al. 2007. TLR3 deficiency in patients with herpes simplex encephalitis. *Science* 317:1522–27
197. Casrouge A, Zhang SY, Eidenschenk C, Jouanguy E, Puel A, et al. 2006. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 314:308–12
198. Zhang S-Y, Casanova J-L. 2015. Inborn errors underlying herpes simplex encephalitis: from TLR3 to IRF3. *J. Exp. Med.* 212:1342–43
199. Bastard P, Manry J, Chen J, Rosain J, Seeleuthner Y, et al. 2021. Herpes simplex encephalitis in a patient with a distinctive form of inherited IFNAR1 deficiency. *J. Clin. Investig.* 131:e139980
200. Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, et al. 2012. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* 491:769–73
201. Zimmer B, Ewaleifoh O, Harschnitz O, Lee YS, Peneau C, et al. 2018. Human iPSC-derived trigeminal neurons lack constitutive TLR3-dependent immunity that protects cortical neurons from HSV-1 infection. *PNAS* 115:E8775–82
202. Gao D, Ciancanelli MJ, Zhang P, Harschnitz O, Bondet V, et al. 2021. TLR3 controls constitutive IFN-β antiviral immunity in human fibroblasts and cortical neurons. *J. Clin. Investig.* 131:e134529
203. Lafaille FG, Harschnitz O, Lee YS, Zhang P, Hasek ML, et al. 2019. Human SNORA31 variations impair cortical neuron-intrinsic immunity to HSV-1 and underlie herpes simplex encephalitis. *Nat. Med.* 25:1873–84
204. Zhang SY, Clark NE, Freije CA, Pauwels E, Taggart AJ, et al. 2018. Inborn errors of RNA lariat metabolism in humans with brainstem viral infection. *Cell* 172:952–65.e18
205. Mancini M, Caignard G, Charbonneau B, Dumaine A, Wu N, et al. 2019. Rel-dependent immune and central nervous system mechanisms control viral replication and inflammation during mouse herpes simplex encephalitis. *J. Immunol.* 202:1479–93
206. Sato R, Kato A, Chimura T, Saitoh SI, Shibata T, et al. 2018. Combating herpesvirus encephalitis by potentiating a TLR3-mTORC2 axis. *Nat. Immunol.* 19:1071–82
207. Bustamante J. 2020. Mendelian susceptibility to mycobacterial disease: recent discoveries. *Hum. Genet.* 139:993–1000
208. Nathan CF, Murray HW, Wiebe ME, Rubin BY. 1983. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* 158:670–89
209. Boisson-Dupuis S, Ramirez-Alejo N, Li Z, Patin E, Rao G, et al. 2018. Tuberculosis and impaired IL-23-dependent IFN-γ immunity in humans homozygous for a common *TYK2* missense variant. *Sci. Immunol.* 3:eaau8714
210. Kerner G, Ramirez-Alejo N, Seeleuthner Y, Yang R, Ogishi M, et al. 2019. Homozygosity for TYK2 P1104A underlies tuberculosis in about 1% of patients in a cohort of European ancestry. *PNAS* 116:10430–34
211. Kerner G, Laval G, Patin E, Boisson-Dupuis S, Abel L, et al. 2021. Human ancient DNA analyses reveal the high burden of tuberculosis in Europeans over the last 2,000 years. *Am. J. Hum. Genet.* 108:517–24
212. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* 259:1739–42
213. Vidal SM, Malo D, Vogan K, Skamene E, Gros P. 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for *Bcg*. *Cell* 73:469–85
214. Casanova JL, Conley ME, Seligman SJ, Abel L, Notarangelo LD. 2014. Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. *J. Exp. Med.* 211:2137–49
215. Israel L, Wang Y, Bulek K, Mina ED, Zhang Z, et al. 2017. Human adaptive immunity rescues an inborn error of innate immunity. *Cell* 168:789–800
216. Drutman SB, Mansouri D, Mahdaviani SA, Neehus A-L, Hum D, et al. 2020. Fatal cytomegalovirus infection in an adult with inherited NOS2 deficiency. *N. Engl. J. Med.* 382:437–45

217. Ciancanelli MJ, Huang SX, Luthra P, Garner H, et al. 2015. Infectious disease: life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. *Science* 348:448–53
218. Hernandez N, Melki I, Jing H, Habib T, Huang SSY, et al. 2018. Life-threatening influenza pneumonitis in a child with inherited IRF9 deficiency. *J. Exp. Med.* 215:2567–85
219. Lim HK, Huang SXL, Chen J, Kerner G, Gilliaux O, et al. 2019. Severe influenza pneumonitis in children with inherited TLR3 deficiency. *J. Exp. Med.* 216:2038–56
220. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, et al. 2020. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* 370:eabd4570
221. Hernandez N, Bucciol G, Moens L, Le Pen J, Shahrooei M, et al. 2019. Inherited IFNAR1 deficiency in otherwise healthy patients with adverse reaction to measles and yellow fever live vaccines. *J. Exp. Med.* 216:2057–70
222. Duncan CJ, Mohamad SM, Young DF, Skelton AJ, Leahy TR, et al. 2015. Human IFNAR2 deficiency: lessons for antiviral immunity. *Sci. Transl. Med.* 7:307ra154
223. Asano T, Boisson B, Onodi F, Matuozzo D, Moncada-Velez M, et al. 2021. X-linked recessive TLR7 deficiency in ~1% of men under 60 years old with life-threatening COVID-19. *Sci. Immunol.* 6:eabl4348
224. Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, et al. 2021. Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. *Sci. Immunol.* 6:eabl4340
225. van der Wijst MGP, Vazquez SE, Hartoularos GC, Bastard P, Grant T, et al. 2021. Type I interferon autoantibodies are associated with systemic immune alterations in patients with COVID-19. *Sci. Transl. Med.* 13(612):eabh2624
226. Zhang Q, Bastard P, Bolze A, Jouanguy E, Zhang SY, et al. 2020. Life-threatening COVID-19: defective interferons unleash excessive inflammation. *Med* 1:14–20
226a. Zhang Q, Pizzorno A, Miorin L, Bastard P, Gervais A, et al. 2022. Autoantibodies against type I IFNs in patients with critical influenza pneumonia. *J. Exp. Med.* 219(11):e20220514
227. García-Sastre A. 2011. Induction and evasion of type I interferon responses by influenza viruses. *Virus Res.* 162:12–18
228. Chen Y, Graf L, Chen T, Liao Q, Bai T, et al. 2021. Rare variant *MX1* alleles increase human susceptibility to zoonotic H7N9 influenza virus. *Science* 373:918–922
229. Van Horebeek L, Dubois B, Goris A. 2019. Somatic variants: new kids on the block in human immunogenetics. *Trends Genet.* 35:935–47
230. Aluri J, Cooper MA. 2021. Genetic mosaicism as a cause of inborn errors of immunity. *J. Clin. Immunol.* 41:718–28