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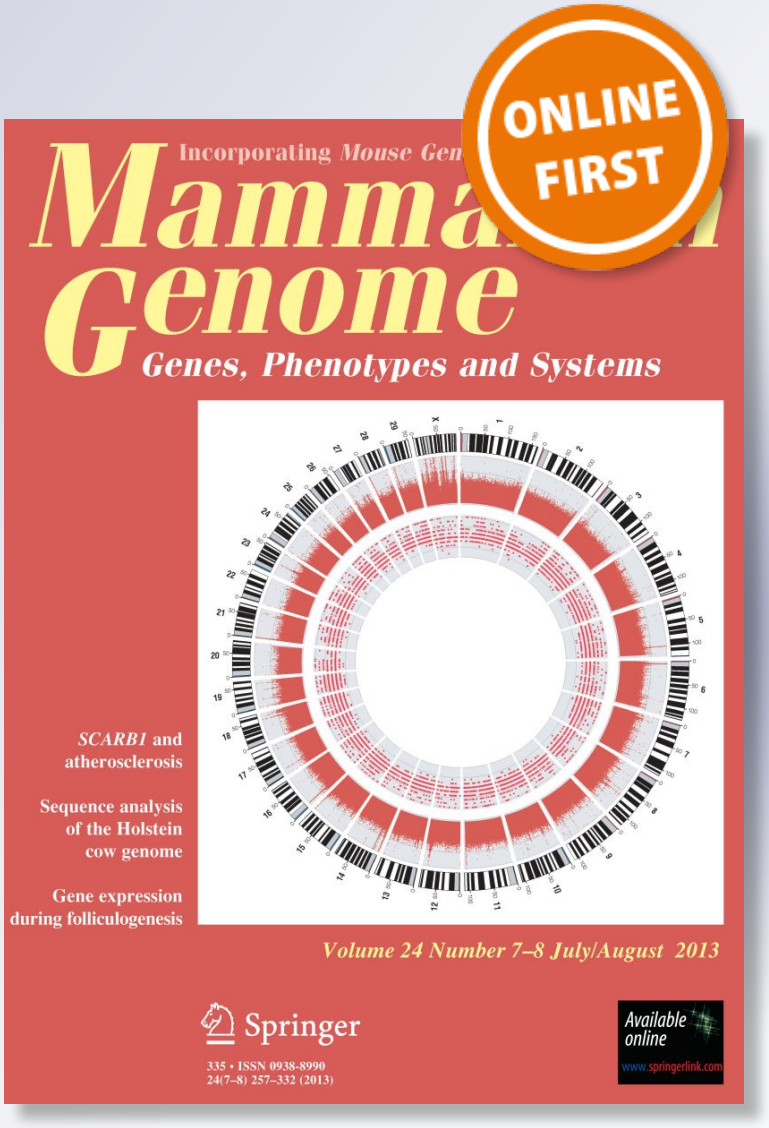
Mammalian Genome

ISSN 0938-8990

Mamm Genome
DOI 10.1007/s00335-018-9766-3



Springer

Mammalian Genome
https://doi.org/10.1007/s00335-018-9766-3

Wild-derived mice: from genetic diversity to variation in immune responses

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Received: 2 April 2018 / Accepted: 23 July 2018

Abstract
Classical inbred mouse strains have historically been instrumental in mapping immunological traits. However, most of the classical strains originate from a relatively limited number of founder animals, largely within the *Mus musculus domesticus* subspecies. Therefore, their genetic diversity is ultimately limited. For this reason, it is not feasible to use these mice for exhaustive interrogation of immune signaling pathways. In order to investigate networks through forward genetic analysis, larger genetic diversity is required than is introduced under laboratory conditions. Recently, inbred strains from other mouse subspecies were established such as *Mus musculus castaneus* and *Mus musculus musculus*, which diverged from a shared common ancestor with *Mus musculus domesticus* more than one million years ago. A direct genomic comparison clearly demonstrates the evolutionary divergence that has occurred between wild-derived mice and the classical inbred strains. When compared to classical inbred strains, wild-derived mice exhibit polymorphisms every 100–200 base pairs. Studying the molecular basis of these traits provides us with insight into how the immune system can evolve regulatory features to accommodate environment-specific constraints. Because most wild-derived strains are able to breed with classical inbred mice, they represent a rich source of evolutionarily significant diversity for forward genetic studies. These organisms are an emerging, though still largely unexplored, model for the identification and study of novel immunological genes.

Genetic approaches to characterization of immune responses

Genetic studies of immune responses have made immense progress due to the advent of genome-wide experimental approaches, such as next-generation sequencing (Rizvi et al. 2017) and CRISPR-based genome editing (Parnas et al. 2015). Nevertheless, with over 24,000 genes in the mouse and human genomes (Sequencing et al. 2002), we still have yet to identify the complete list of genes involved in the host immune response, due to the challenge of assigning a functional role to each gene product (Eppig et al. 2015). Furthermore, identifying the function of immune genes has been complicated by the fact that many—if not all—of these genes have multiple functions and participate in gene–gene interactions (White et al. 2009).

Addressing these questions has occupied immunologists for decades. However, as the tools of molecular biology continue to improve, so do our techniques for identifying and characterizing genes important in the immune response. Identification of the earliest characterized inflammatory genes, such as the interferon (IFN) (Watanabe 2004), Interleukin-1 (IL-1) (Dinarello 1994), and Tumor necrosis factor alpha (TNF) (Beutler et al. 1985), relied on biochemical purification, and the protein products were known well before their genes were cloned. Some key immune effectors are still found by means of biochemical purification, as exemplified by the discoveries of the role of 3′ repair exonuclease TREX1 and STING (Stimulator of Interferon Genes) in responses to cytosolic DNA. TREX1 was purified biochemically based on its physical interaction with biotinylated DNA (Stetson and Medzhitov 2006) while STING (encoded by *TMEM173*) was identified via its interaction with Major histocompatibility complex class II (MHC-II) (Jin et al. 2008). A strict nucleic acid-specific approach was used in the identification of the class-switch

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Published online: 28 July 2018

recombination and somatic hypermutation inducing factor activation-induced cytidine deaminase (AID) by subtractive cDNA hybridization (Muramatsu et al. 1999). In addition, bioinformatics approaches are increasingly being utilized to identify important immune genes. The IL-23p19 subunit, which was identified based on its homology to IL-12p35, is an example of a gene uncovered through this approach. Identification of IL-23p19 provided the foundation for the discovery of the Th17 T cell subset, which constituted a dramatic leap forward in our knowledge of T cell-mediated immunity (O'Shea et al. 2009).

Despite the significant advances achieved by these methods, traditional genetic approaches—reverse genetics and forward genetics—have been predominantly used to reveal the function of genes involved in the immune response. Reverse genetics, which is a "gene first" approach, has played an indispensible role in characterizing the immune response (Curtis 2004). An a priori hypothesis is generated regarding a potential function of a known gene, and this gene is subsequently targeted for experimental manipulation. Genes can be knocked out, or they can be overexpressed after they are introduced into a unique site in the genome. Targeting of many Toll-like receptors (TLRs) and the known signaling components of TLR-pathways in mice have provided a wealth of information about the specific role that each gene plays in the inflammatory response (Akira and Takeda 2004).

In contrast, forward genetics identifies a causative link between a gene and an observed phenotype via an unbiased "phenotype first" approach when the identity of the gene is not known (Beutler et al. 2003). This method exploits phenotypic variations between individuals (Casanova et al. 2002), and uses the genetic diversity of these individuals to identify a correlation between a phenotype and a genomic area (locus) responsible for the phenotype. Genetic diversity between individuals, or between mouse strains can occur naturally or can be experimentally introduced through mutagenesis. Forward genetic analyses have led to remarkable advances in our understanding of innate immune function (Hoebe and Beutler 2005), helping in some cases to resolve a long-standing paradigm such as the identification of TLR4 as the lipopolysaccharide (LPS)-sensor (Poltorak et al. 1998). Forward genetic techniques can also help to identify additional unexpected functions for genes previously believed to be involved in completely unrelated activities. For example, the ATP-sensitive potassium channel Kir6.1—formerly understood to play a role in vascular contraction and energy homeostasis (Yokoshiki et al. 1998)—was found to be a key effector in host defense against cytomegalovirus (Croker et al. 2007). Often, forward genetics helps to further characterize functions for previously well-characterized genes such as CD14, which in addition to being a co-receptor for LPS, was found to be involved in the recognition of a distinct "smooth" LPS (Jiang et al. 2005).

To execute forward genetic approaches in mouse strains exhibiting a phenotypic difference, parental strains are mated to produce an F1 progeny, followed by an intercross of F1 or backcross to the parental strain with recessive inheritance for the trait. With sufficient cohort size, the genetic diversity generated in the second-generation progeny (F2 or N2) will allow for correlations to be made between phenotype and genotype, leading to the identification of a potential causative locus. Once a locus is identified and confirmed, candidate genes in the region can be examined, or more recombination events (i.e., more meioses) can be generated to narrow the interval with the highest linkage until only one or a few genes remain to be tested for their role in the phenotype (Hoebe et al. 2006).

Mapping strategies are usually quite straightforward for dichotomous traits conferred by variation in single genes. However, mapping quantitative traits (Mott and Flint 2013)—such as the concentration of cytokine produced in response to TLR stimulation—that are often under the control of numerous genes, requires a slightly more complex strategy. Quantitative trait locus (QTL) analysis was designed to assign linkage scores to loci based upon their association with statistically significant deviation from the mean in a sample set (Peters et al. 2007). This approach has greatly facilitated the mapping of complex traits, and has been used in studies of such diverse phenotypes as serum cholesterol levels and anxiety. While QTL analysis has been extremely useful at identifying statistically significant associations, it is difficult to use QTL for narrowing genetic intervals associated with these traits because of their polygenic nature. Due to these complications, candidate genes are often identified from large genomic loci identified by QTL for investigation (Shmulewitz et al. 2006). Numerous criteria can be used to pick gene candidates, including known roles in the phenotype of interest. Gene expression analysis has been used in combination with QTL-based mapping as a powerful tool to identify gene expression polymorphisms that underlie complex traits (Wayne and McIntyre 2002). Recently established searchable website (mousepost.be) provides a list of sequence variation in 36 mouse inbred strains including wild-derived strains (Timmermans et al. 2017). Using this site one could easily verify the presence of deleterious mutations in gene candidates from the linked with the trait area. Another useful resource for investigation of how genetic variation affects cellular phenotypes is recently published comparison of >50 million variations in different strains of mice—including wild-derived strains—on mRNA and nascent transcription (Link et al. 2018). The study revealed that many of the polymorphisms act in cis to affect transcription factor binding and gene expression.

Wild-derived mice: a reservoir of novel immune phenotypes

The forward genetic analysis begins with identification of phenotypic variants within a population or between mouse strains. Classical inbred mouse strains have historically been instrumental in mapping immunological traits. However, most of the classical strains originate from a relatively limited number of founder animals, largely within the *Mus musculus domesticus* subspecies, and their genetic diversity is ultimately limited (Frazer et al. 2007; Yang et al. 2007). Therefore, it is not possible to use these mice for exhaustive interrogation of immune signaling pathways. Because of this inherent limitation in readily available inbred strains, the genetics of innate immunity has been increasingly studied by other means, such as genome-wide ENU mutagenesis, which has resulted in numerous remarkable discoveries of gene functions. Comprehensive ENU screens currently in use can achieve almost complete coverage of the mouse genome, and result in functional impairment of most non-embryonic lethal genes (Hoebe et al. 2006).

Despite their extensive genomic coverage, ENU screens still do not tap the entire wealth of information that can be uncovered using forward genetics. Although a mutagenesis approach is well suited to examine the functional consequence of a single genetic lesion, it does not allow for comparison of unique multi-gene interactions that arise in the context of an evolved gene network. It is increasingly recognized that immune signaling pathways such as the TLR response rely on complex interactions between gene products rather than a linear series of signaling events (Oda and Kitano 2006). Indeed, when the interaction between genes plays a critical role in a phenotype, the genes often co-evolve under selective pressure, and can be identified by comparing evolutionarily distant individuals (Conner et al. 2009, 2008). In order to investigate the networks through forward genetic analysis, larger genetic diversity is required than is introduced under laboratory conditions (Ishikawa 2013).

All members of the *Mus musculus* species arose from a common ancestor more than one million years ago. Three major subspecies then diverged (Gregorova et al. 2008), primarily along geographic boundaries, to comprise the *Mus musculus musculus* (eastern Europe, Russia, China), *Mus musculus castaneus* (western and southeast Asia, China), and *Mus musculus domesticus* (western Europe) subspecies (Phifer-Rixey et al. 2014). *Mus musculus molossinus*, a hybrid between *musculus* and *castaneus* subspecies, arose in Japan approximately 10,000 years ago and is now considered by some to be a fourth subspecies. Animals from "fancy mouse"(Koide et al. 1998) breeders in Europe and Asia were interbred several 100 years ago, which resulted in a mixing of subspecific genomes (Liu et al. 2008). These mice were the ancestors of the founders employed by Ms. Lathrop and others, and were used to generate our current repertoire of classical inbred mouse strains for scientific research (Guenet and Bonhomme 2003). Thus, classical inbred strains have a somewhat assorted contribution of genomic regions from several mouse subspecies.

Two approaches of subspecific contribution analysis identify that a majority of the genome of classical inbred strains such as C57BL/6 is derived from the *Mus musculus domesticus* subspecies, calculated at 68% in one study and 94% in the second, while *Mus musculus castaneus* and *Mus musculus musculus* contribute less than 10% (Frazer et al. 2007; Yang et al. 2011). Although interspecific recombination also exists in wild-derived mice (such as MOLF/Ei), the contribution of *Mus musculus domesticus* is significantly lower, calculated at 11% of the genome (Yang et al. 2007). In MOLF/Ei mice, *Mus musculus castaneus* comprises 15% of the genome and *Mus musculus musculus* makes up 74%. This comparison clearly demonstrates the evolutionary divergence that wild-derived mice contain relative to classical inbred strains. When compared to commonly studied inbred strains, wild-derived mice exhibit polymorphisms every 100–200 base pairs (Ideraabdullah et al. 2004). Importantly, the genetic differences between subspecies have arisen in the process of subspeciation, during which subspecies were separated in a strong evolutionary context and exposed to different pathogens, and were therefore selected for specific traits. Studying the molecular basis of these traits provides us with insight into how the immune system can evolve regulatory features to accommodate environment-specific constraints. Because most wild-derived strains are able to breed with classical inbred mice, they represent a rich source of evolutionarily significant diversity for forward genetic studies. These organisms are an emerging, though still largely unexplored, model for the identification and study of novel immunological genes. In support of further investigation of genetic diversity of the wild-derived mice, we provide below several examples of genetic screens interrogating immune responses in these mice.

Spretus

Inbred strains derived from *Mus spretus* have the highest genetic diversity among all wild-derived strains because this species is one of the most distant relatives of the laboratory strains (predating the *Mus musculus* species) that can still breed with laboratory strains to produce fertile hybrids. The strain SPRET/EiJ was derived from wild animals trapped in Santa Fe (near Granada, Spain) by L. Thaler in 1978, then propagated as a closed colony by F. Bonhomme, and then

in Institut Pasteur since 1986. Extensive genetic polymorphisms in *Mus spretus* have ensured its widespread use in many areas of genetics (Dejager et al. 2009) but also was the reason of sterility in F1 (C57BL/6 × SPRETUS) hybrids males. This problem has been recently overcome using in vitro recombination (Lazzarano et al. 2018). According to this approach, random mitotic crossovers were induced in the embryonic stem cells from F1 mice, which facilitated mapping and gene identification in 21 days. SPRET/EiJ mice have been extremely helpful in evolutionary studies of genomes and speciation. Specifically, they were found to be resistant to LPS-induced septic shock. In addition, they are also resistant to TNF-induced lethality in vivo, since TNF is the main effector of LPS-shock (Staelens et al. 2002). The trait was inherited in a dominant manner for SPRET/EiJ, as F1 hybrids are largely also resistant. An interspecific backcross experiment between C57BL/6 and SPRET/EiJ mice revealed that the TNF hypo-response is linked to loci on chromosomes 2, 6, and 11 (Staelens et al. 2002).

In a follow-up study, the same group demonstrated that SPRET/EiJ mice exhibit resistance against LPS-induced lethality (Mahieu et al. 2006). The trait was transmitted in a dominant manner, and was mediated by bone marrow-derived cells. Macrophages from these mice exhibit normal MyD88-dependent signaling, but defective IFN-beta signaling, resulting in low IFN production. Furthermore, IFN induction by LPS or influenza virus was low in SPRET/EiJ mice, but IFN-beta treatment restored sensitivity to LPS. Stimulation of SPRET/EiJ macrophages led to rapid down-regulation of IFN type 1 receptor mRNA expression, which was revealed in poor induction of IFN-beta-dependent genes. This finding indicated that the resistance of SPRET/EiJ mice to LPS was due to disruption of a positive-feedback loop that amplified IFN-beta production.

The LPS resistance in SPRET/EiJ mapped to the distal region of the X-chromosome (Pinheiro et al. 2013). The GR-inducible gene *Tsc22d3* (encoding the protein GILZ) located in the critical region on the X-chromosome, showed a higher expressed SPRET/EiJ allele, regulated in cis. Higher GILZ levels were causally related to reduced inflammation, as shown with knockdown and overexpression studies in macrophages. Transient overexpression of Gilz by hydrodynamic plasmid injection confirmed that Gilz protects mice against endotoxemia. These data strongly suggested that Gilz was responsible for the LPS resistance of SPRET/EiJ mice, and might be targeted for treatment in sepsis.

Cast

Infection with monkeypox virus (MPXV) causes disease manifestations in humans that are similar, although usually less severe, than those of smallpox. In a genetic screen for susceptibility to MPXV, Americo et al. screened 38 inbred mouse strains (Americo et al. 2010) including the wild-derived strains. Three highly susceptible wild-derived inbred strains were identified including CAST/EiJ mice, which exhibited weight loss, morbidity, and death in a dose-dependent manner. Immunization with vaccinia virus fully protected mice from lethal doses of MPXV, resulting in induction of antigen-specific T- and B-lymphocyte responses. The new mouse model helped to evaluate potential vaccines and therapeutics.

Further comparison of virus dissemination and induced cytokine production between susceptible CAST/EiJ and resistant BALB/c mouse strains revealed a rapid and efficient spread of virus to internal organs in CAST/EiJ mice, while virus remained largely restricted to the lungs in BALB/c mice (Earl et al. 2012). Induction of IFN-gamma and CCL5 played an important role in protection against the virus, as addition of exogenous IFN-gamma protected CAST mice, and inactivation of the IFN-gamma gene made C57BL/6 mice susceptible to virus (Earl et al. 2012).

The increased sensitivity of CAST relative to C57BL/6 was confirmed for other members of the family such as vaccinia virus (VACV) and cowpox virus (CPXV) (Americo et al. 2014), whereas there was little difference in the susceptibility of the mouse strains to herpes simplex virus, another DNA-virus. Using bioluminescence imaging, the authors confirmed rapid viral replication of MPXV, and dissemination to lungs and abdominal organs in CAST mice (Earl et al. 2015). In a follow-up genetic inquiry using CAST and C57BL/6 mice, the authors concluded that the resistance of C57BL/6 to infection is a dominantly transmitted trait, as F1 (CASTxC57BL/6) were relatively resistant. Crossing F1 mice back to C57BL/6 revealed variable phenotype, suggesting that several loci may be responsible for conferring the trait (Earl et al. 2015a, b).

Genetic studies of host response to gram-negative bacteria infection in MOLF/Ei mice

It has been well established that host response to gram-negative bacteria is genetically controlled. Inbred mice infected with the gram-negative bacterium Salmonella typhimurium exhibit a wide range of susceptibility to infection, with the wild-derived strain MOLF/Ei being extremely susceptible (Sebastiani et al. 2002). The kinetics of bacterial proliferation in different tissue sections suggested that MOLF/Ei mice were not overwhelmed by bacterial growth, but rather had a unique cytokine response, characterized by strong upregulation of NF-kB and TLR-2-mediated signaling. Previous analysis of the genetic basis of resistance or susceptibility to infection with S. typhimurium in MOLF/Ei using an F2 intercross between C57BL/6J and MOLF/Ei identified

several loci that conferred either susceptibility or protection to infection (Sebastiani et al. 1998). The locus on chromosome 1 (termed *Ity3* Immunity to Typhimurium) conferred susceptibility to infections, whereas a locus on chromosome 11 (*Ity2*) was protective. The recessively inherited *Ity3* is located in a genomic region spanning 84 Mb that is rich in genes, among which is *Ncf2* (encoding p67phox, a subunit of the multiprotein enzyme complex NADPH oxidase) which was prioritized based on analysis of expression, function, and sequencing (Sancho-Shimizu and Malo 2006). Both *Ity2* and *Ity3* loci were isolated using congenic lines, which were used to define the critical intervals underlying Ity2 and Ity3, and to identify candidate genes encoded by *Ity2 (Havcr2)* and *Ity3 (Chi3l1)*(53) (Sancho-Shimizu et al. 2007). A novel cross between MOLF/Ei and the resistant strain 129S helped to fine-map *Ity2* and identify a novel locus on chr 13 *Ity13* (Khan et al. 2012). Further transcriptional profiling helped to reveal the role for both IFN-I signaling and TRP53 signaling in the pathogenesis of Salmonella infection (Khan et al. 2012). Fine mapping of the Ity3 locus revealed another locus encoding gene candidate *Selp*, that in addition to *Ncf2* contributed to the trait (Khan et al. 2014). SELP-deficient mice confirmed the effect of the gene in the host response to the infection. To further define the loci, and to characterize the in vivo contribution of these loci to the trait, 12 recombinant congenic were generated on the C57BL/6J background. Studies of survival and bacterial burden provided support that several factors within Ity3 contribute to the immune response to Salmonella infection in MOLF/Ei mice (Khan et al. 2014a, b).

Regulation of hyper-responsiveness to TLR stimulation in MOLF/Ei

The response to TLR stimulation in MOLF/Ei mice is characterized by a more potent acute phase compared to C57BL6 (Conner et al. 2009). In macrophages, stimulation of TLR2 by lipoteichoic acid (LTA) induces increased IL-6 production in MOLF/Ei compared to C57BL/6. In order to determine the genetic cause for the variation in response to TLR2 stimulation, an N2 panel was generated between MOLF/Ei and C57BL/6 mice. Using macrophages from this panel, heightened IL-6 production was mapped to the *Why1* locus, a 21-Mb region located on Chromosome 6. Knockdown experiments confirmed that the trait is conferred by *Irak2* (Interleukin 1 receptor-associated kinase 2), which is usually expressed as several isoforms of IRAK2—one of which is anti-inflammatory—except in MOLF/Ei, in which the isoform is conferred early and leads to more potent activation of NF-kB and p38 kinase. Crucially, *Irak2* is in epistatic interaction with other loci, one of them encoding TIRAP (TIR domain-containing adaptor protein), which is an essential component of the TLR-initiated signaling complex.

To terminate the overproduction of inflammatory cytokines, MOLF/Ei—as well as some other wild-derived mice, but not classical inbred mice—upregulate IRAK1BP1 an inhibitor of NF-kB. IRAK1BP1 was identified in the same N2 screen used to identify IRAK2, and is found within the *Why2* locus on Chromosome 9 (Conner et al. 2008). Upon upregulation of IRAK1BP1, levels of IL-6 decrease but levels of IL-10, an anti-inflammatory cytokine, increase. IRAK1BP1 facilitates its anti-inflammatory role by promoting nuclear translocation of p50 homodimers, which is the inhibitory component of NF-kB (Conner et al. 2010).

Responses to bacterial DNA in MOLF/Ei

In a genetic screen for responses to bacterial DNA, MOLF/Ei macrophages were poorly responsive to CpG DNA. Using a N2 cross [F1(C57BL/6xMOLF/Ei)xMOLF/Ei], the trait was mapped to a 1 Mbp region on Chromosome 2 encoding MRC1 (mannose receptor, CD206). MOLF/Ei macrophages are hypo-responsive to CpG due to low levels of MRC1, which is important for phagocytosis of DNA in MOLF/Ei but not in C57BL/6 macrophages (Moseman et al. 2013). Interestingly, MOLF/Ei B cells proliferate but do not produce any cytokines in response to CpG as compared to C57BL/6 cells. Furthermore, MOLF/Ei pDCs (plasmocytoid dendritic cells) produce TNF and IL-6 in response to type B CpG, which is in striking contrast to pDCs from C57BL/6 mice, which are essentially non-responsive to this type of CpG (not published).

Resistance to liver failure in MOLF/Ei and MSM/Ms Mice

The MSM/Ms mouse strain was generated in Mishima city, Shizuoka prefecture, Japan from wild-caught mice of the *Mus musculus molossinus* subspecies (Moriwaki et al. 2009). Breeding for the strain began in 1978, after six mice were donated to the Cytogenetics Department of the National Institute of Genetics. Similar to other wild-derived strains, MSM/Ms provides a rich reservoir for genetic diversity compared to the classical laboratory mouse strains.

We established a novel model of in vivo resistance to death receptor-mediated lethality in several strains of wild-derived mice including MSM/Ms and MOLF/Ei. Specifically, MOLF/Ei and MSM/Ms mice are profoundly resistant to ligation of Fas via Jo2 antibodies. Linkage analysis in F2 intercross (C57BL/6 × MSM/Ms) progeny complemented with high-throughput RNA-sequencing identified a 21-bp insertion in the 3′ UTR of the fifth exon of *Cflar* in MSM/Ms that influences differential splicing of cFLIP mRNA (Ram et al. 2016). Intriguingly, we observed that MSM/Ms liver cells predominantly express the long cFLIP variant, in contrast to C57BL/6 liver cells, which have higher levels of

short cFLIP. Unlike short cFLIP, long cFLIP forms an enzymatically active heterodimer with CASP8, which cleaves RIP1, thus preventing induction of apoptosis.

CYLD regulates the balance between inflammation and cell death

In a viability screen of peritoneal macrophages from an N2 panel (C57BL/6xF1(C57BL/6xMOLF/Ei), resistance to TLR-mediated necroptosis in MOLF/Ei macrophages was mapped to a 5.1 Mb locus on chromosome 8. Due to its previously established role in cell death, CYLD (cylindromatosis), which confers resistance to TLR-induced necrosis (Schworer et al. 2014) was selected as the most likely gene candidate. The resistance of MOLF/Ei macrophages to necroptosis was conferred by relatively low levels of CYLD mRNA in MOLF/Ei as compared to C57BL/6.

Immune responses to cytosolic DNA in MOLF/Ei

In a screen performed in macrophages from an F2 panel (C57BL/6xMOLF/Ei), we observed attenuated IFN production in response to cytosolic DNA in MOLF/Ei macrophages. Through high-resolution mapping, *Tmem173*, encoding STING was identified. We discovered novel mutations in the N-terminal domain of *Sting* in the MOLF/Ei strain, which also regulated in vivo responses to HSV and Listeria monocytogenes (Surpris et al. 2015). These mutations are responsible for low levels of IFN-beta, caused by failure of MOLF/Ei STING to translocate from the endoplasmic reticulum. Strikingly, the IFN-defect is complemented with overproduction of IL-6 in MOLF/Ei, a crucial functional difference not apparent in classical inbred mice. Thus, understanding the functional significance of polymorphisms in MOLF/Ei STING can provide basic mechanistic insights relevant to humans.

Conclusion

These provided examples demonstrate the ability of an unbiased phenotypic screen in evolutionarily diverse mouse subspecies to complement biochemical studies of immune responses. Although the genetic diversity of these mouse strains has been appreciated previously, it has also limited their use in forward genetic analyses because of the often polygenic nature underlying observed traits. In addition, high levels of polymorphism in these mice, as compared with classical inbred strains, have made it difficult to establish a causative link between a candidate-gene and a phenotype. Although not a subject of the current review, the diversity of the wild-derived strains has been utilized in the establishment of the Collaborative Cross (Maurizio and Ferris 2017) and the Diversity Outbred (Churchill et al. 2012) programs by mouse geneticists at Jackson Laboratories.

Funding Funding was funded by National Institute of Allergy and Infectious Diseases (Grant Nos. AI119833, AI126050, AI126489 to A.P) and Russian Science Fund Project 15-15-00100.

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