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Recently, the Centre for Immunity, Infection and Evolution sponsored a one-day symposium entitled “Wild Immunology.” The CIIE is a new Wellcome Trust–funded initiative with the remit to connect evolutionary biology and ecology with research in immunology and infectious diseases in order to gain an interdisciplinary perspective on challenges to global health. The central question of the symposium was, “Why should we try to understand infection and immunity in wild systems?” Specifically, how does the immune response operate in the wild and how do multiple coinfections and commensalism affect immune responses and host health in these wild systems? The symposium brought together a broad program of speakers, ranging from laboratory immunologists to infectious disease ecologists, working on wild birds, unmanaged animals, wild and laboratory rodents, and on questions ranging from the dynamics of coinfection to how commensal bacteria affect the development of the immune system. The meeting on wild immunology, organized by Amy Pedersen, Simon Babayan, and Rick Maizels, was held at the University of Edinburgh on 30 June 2011.

**Introducing wild immunology**

In the wild, organisms face many pressures (e.g. parasites, pathogens, commensal organisms, seasonal-

ity, resource availability) that affect their health and fitness. A great deal of our knowledge on infection and immunity, however, has been developed in highly controlled laboratory settings where variation is minimized to more easily identify molecular and cellular immune mechanisms. While traditionally maintaining strong connections between the lab and the field, greater practical achievements in human medicine and veterinary medicine have been difficult because of the challenges of associating detailed mechanistic interventions developed in the lab with what has been learned in the field about the effects on host fitness in natural settings. The aims of the conference on wild immunology were to bring these approaches together, to address shared questions about the role of the immune system in natural populations, and to better integrate laboratory-based immunology into the actual health of humans and animals.<sup>1</sup>

Judi Allen (professor of immunobiology, University of Edinburgh) introduced the topic of wild immunology from her perspective as a laboratory-based immunologist. She argued that wild immunology draws from both ecological immunology (eco-immunology) and laboratory-based immunology, and aims to link immunity and infection with host health and fitness in wild systems.<sup>1</sup> Specifically, Allen said, wild immunology is “about

communicating between lab studies and studies in *wild* systems.”

The burgeoning field of eco-immunology has focused on understanding costs and trade-offs in an organism’s life-history and fitness associated with mounting immune responses (see, for example, Refs. 2 and 3). Many studies in this field have aimed to identify a single measure of the immune response (e.g., white blood cell number<sup>4</sup> or spleen size<sup>5</sup>), termed *immunocompetence*, as a meaningful metric of immune function and the relationship of immune function to host fitness or disease burden. This approach may be counterintuitive to those laboratory immunologists who instead seek to understand the complex network of all, or the majority, of the mechanisms that underlie immune functions in general. The link and yet greatest difference between immunologists and ecologists is in their approach to variation (e.g., host genotype, parasite genotype, sex, seasonality, coinfection, and commensal community); laboratory immunologists aim to minimize variation (“noise”) in the systems they study, while ecologists focus squarely on natural variation. Generally, ecologists have created a toolbox with which to understand and explain variation in wild systems, and this is often at the heart of their research. In contrast, laboratory immunologists intentionally reduce variation by controlling, for example, the genetics, environment, and resources in experimental systems in order to pinpoint important underlying mechanisms. Allen suggested that it is time for both fields to “get real,” that is, ecologists need more specific measures of immune competence that are based on *real* mechanisms of immunity and resistance, while laboratory immunologists need to get out of the lab to understand if the mechanisms they define in controlled lab systems are *real*—meaningful—for health and fitness of wild systems.

Collaborations across this divide can provide unique and valuable insight (for example, see Refs. 1 and 6). Currently, however, several barriers exist that prevent successful interdisciplinary communication, such as the different languages and approaches employed by ecologists and laboratory immunologists. Allen suggested, among other things, that an important divide separating ecologists and laboratory immunologists is the ways they write and read scientific literature. For example, most ecological/evolutionary studies provide essential informa-

tion on the study design, with extensive reference to the statistical/modeling approaches undertaken; such information is a central element of Materials and Methods sections of papers in this field, with ecologists scouring these sections. By contrast, laboratory immunologists often read Materials and Methods sections of manuscripts only if they want to know the details of a particular experiment, and typically they do not consider which statistical methods were employed as central to the paper. Additionally, there are basic language and communication differences that make successful crosstalk difficult. For example, ecologists define *tolerance* as a host’s ability to limit damage caused by a given parasite density.<sup>7–9</sup> In contrast, laboratory immunologists define tolerance as the process (or processes) by which immune responses (to an antigen, including self-antigens) are negatively regulated;<sup>10</sup> thus, a weaker immune response can be associated with lower risk of immunopathology. In such counterintuitive cases, especially, proactive discussions between ecologists and laboratory immunologists are likely to prove the most compelling and productive.

### From the field to the lab

The next talk by Jan Bradley (professor of parasitology, University of Nottingham) focused on using wild rodents as model systems for understanding mammalian immune systems in a naturally variable world. Humans and wild animals live in variable environments and are exposed to, and often infected by, a diverse array of commensals, parasites, and pathogens throughout their life. Bradley pointed out that, in humans, coinfections can modulate immune responses and affect regulatory processes.<sup>11</sup> Yet, Bradley suggested that understanding how variation affects immune responses in humans is difficult because of ethical and financial issues. However, she highlighted that by adapting the tools and techniques from laboratory-based immunological studies on inbred mice to wild rodents may be a useful avenue for understanding how variation affects immunity.<sup>12</sup> Specifically, measuring immune phenotypes, which will reflect immune responsiveness and function, of wild rodents within the context of their environments, genotypes, and pathogens may provide an excellent opportunity to uncover how the mammalian immune system responds to diverse infections in the natural environment.

In the first part of her presentation, Bradley discussed recent research on innate immune responses in wild rodents, specifically of the wood mouse *Apodemus sylvaticus*, a native rodent of UK woodlands. Using a cross-sectional study design, Bradley and colleagues collected wild wood mice, assessed their macro- and microparasite infections, and conducted a series of innate immunological analyses. Specifically, they used a panel of Toll-like receptor (TLR) agonists to stimulate immune responses in splenocytes, the surrogate marker for which was production of tumor necrosis factor alpha (TNF- $\alpha$ ). They found that burdens of the intestinal nematode *Heligomosomoides polygrus* and the louse *Polyplax serrata* were negatively correlated with TNF- $\alpha$  production.<sup>13</sup> By contrast, the response of intestinal protozoan microparasites *Eimeria spp.* to TLR agonist stimulation was significantly positively associated with TNF- $\alpha$  production, the most important TLR receptors being TLR7 and TLR9.<sup>13</sup> Such TLR-mediated responses, likely meaningful indicators of innate responsiveness in general,<sup>14</sup> may be an important tool for understanding the immunomodulatory effects of parasites in wild systems. Bradley and colleagues are now building on these results from wild mice by working back in the lab using multiple low-dose (“trickle”) nematode infections to better model real-world exposure in lab mice.

The next part of the talk focused on investigating the immune responses of an extensively studied population of wild field voles (*Microtus agrestis*) in Kielder Forest in Northern England. The vole populations fluctuate on a 3- to 4-year cycle,<sup>15</sup> and much is known about their microparasite (e.g., Cowpox virus,<sup>16</sup> *Trypanosoma microti*,<sup>17</sup> *Bartonella spp.*,<sup>18</sup> and *Anaplasma phagocytophilum*<sup>19</sup>) and macroparasite<sup>20</sup> communities. Again using a cross-sectional study design, Bradley and colleagues measured several cellular assays of pro- and anti-inflammatory signaling responses (both transcription factors and cytokines) quantified by Q-PCR. They found striking variation in immune measures among the field voles, specifically that temporal patterns (i.e., seasonality) and life history stages (i.e., reproduction) strongly affected immune expression.<sup>21</sup> Strong negative associations between inflammatory mediators and measures of condition (i.e., liver size, spleen size, and splenocyte number) were also found. Using a grouping analysis, Bradley and colleagues found that measures of pro-

and anti-inflammatory responses were highest in the winter but differed in summer when the minimum responses were found.<sup>21</sup>

Bradley highlighted that these novel approaches will help build useful immunological tools and techniques to begin to understand the variation in immune phenotypes found in wild populations. By integrating the various methods (measuring life history, parasite infections, environmental variation, and immune phenotypes) within both observational and, eventually, experimental approaches, we will begin to understand what factors drive immune phenotypes and parasite burdens in wild populations. Bradley concluded by suggesting that wild immunology will be a very useful avenue that links the laboratory mouse with the real world variation seen in human populations.

### Immune potency of wild mice

Mark Viney (professor of zoology, University of Bristol) presented a comparative study of immune function in wild and laboratory mice. Viney suggested that the immune responses of wild animals have been rather poorly studied despite the fact that lab and wild rodents often vary in their response to parasites and pathogens.<sup>1</sup> He highlighted that the immune function of wild animals may differ from that of laboratory-bred animals because of their different environments. Viney contends that this idea follows from the concept in life history theory called *resource partitioning*, in which animals distribute scarce resources to all aspects of life, including costly immune responses. Therefore, well-resourced laboratory animals in benign, or controlled, environments may have substantially greater resources to direct to immune function than wild mice, which live in demanding environments where resources can be much more limited.<sup>22</sup> A logical extension of this idea is that there may be substantial inter-individual variation in the immune function of wild animals because different individuals acquire different amounts of resources and have different demands on expenditure of those resources.

To investigate this idea, Viney and colleagues compared the immune function of a laboratory bred mouse strain (C57BL/6, a widely used strain that has been maintained for many decades) and wild-caught *Mus musculus*.<sup>23</sup> Specifically, they compared the immune responses of lab and wild-caught mice to a novel antigen with which they were

immunized and found that, by most immune measures, wild-caught mice produced higher concentrations and more avid antigen-specific IgG responses, as well as higher concentrations of total IgG and IgE, compared with laboratory-bred mice. Viney also analyzed cell populations by flow cytometry, which demonstrated a comparatively greater overall level of activation of T helper cells, macrophages, and dendritic cells in wild mice, but no differences in regulatory T ( $T_{reg}$ ) cell activation.<sup>23</sup> Importantly, they observed that these immune measures were substantially more variable among wild-caught mice than among the laboratory-bred mice (as in Ref. 24). Thus in contrast to their original hypothesis about limited resources in the wild leading to poorer immune function, these results suggest that wild mice have stronger and more varied immune responses, despite being less than two-thirds the mass of lab mice and having higher levels of leptin (a hormone involved in energy expenditure and metabolism).

The next challenge of this research will be to understand which aspects of an individual animal's life determines its immune function in the wild and what factors lead to the extreme variation found across individuals. Lastly, Viney pointed out that the study of wild immunology is important because it allows us to link measures of immune function with host health and fitness in natural settings and expands the current taxonomically restricted set of laboratory animals used in immunological studies.

### Cytokine polymorphisms in wild populations

Steve Paterson (senior lecturer, University of Liverpool) presented a study linking polymorphisms in cytokine-coding genes with immune responses and pathogen resistance in wild rodent populations. His research was motivated by a major question in wild immunology: why do individuals vary in their immune responses and resistance to pathogens and parasites? Many of the sources of this variation may be environmental, such as nutrition, microbiota (microorganisms inhabiting the gastrointestinal tract), and coinfection.<sup>1,23</sup> Equally, however, an important source of variation in immune responses and resistance is likely to be due to genetic differences among individuals. In support of this, some individuals in human populations appear to be far more susceptible to helminth reinfection than

others, which may be correlated to distinctive immunological phenotypes.<sup>25–27</sup> Quantitative genetic analysis of parasite infection and reinfection also highlights a strong genetic component to susceptibility.<sup>28,29</sup>

However, until now, genetic studies of immune response and resistance in natural populations have been limited almost entirely to the major histocompatibility complex (MHC).<sup>30,31</sup> This is an important, highly polymorphic component of the vertebrate immune system required for antigen presentation. Paterson highlights that despite numerous studies having demonstrated balancing selection from sequence and allele frequency data, and linked individual variation to differences in pathogen resistance, they have been almost exclusively based on the MHC. However, the MHC only contributes a small proportion of the genetic variation in resistance to infectious disease in mammals.<sup>32</sup> Indeed, the MHC is only a subset of the genes involved in the immune system, and Paterson and colleagues argue that molecular ecologists have been somewhat narrow in not looking for functional variation elsewhere in the genome with respect to pathogen resistance. Because of this, molecular ecology lags behind human genetics, where a far broader range of candidate loci are considered and where genome-wide association studies (GWAS) are now increasingly being deployed to understand the genetic determinants of pathogen resistance and immunity.<sup>33</sup>

Cytokines are key signaling molecules of the immune system, and genetic polymorphisms in cytokine-coding genes have repeatedly been implicated in resistance to pathogens in humans.<sup>34–36</sup> As such, they potentially provide a group of genes that may underlie variation in resistance to a wide range of infections in the natural environment. Paterson used the same wild vole system as described above by Bradley, where individuals live in natural conditions and are likely exposed to a diverse array of parasites. His group conducted a single nucleotide polymorphism (SNP) analysis of the cDNA produced from a wide spectrum of cytokine genes to identify signatures of selection acting on these polymorphisms, and to associate cytokine polymorphisms with variation in immunological parameters and pathogen resistance. They found that two cytokine genes, *Il1b* and *Il2*, showed evidence of balancing selection acting on both DNA sequence and allele frequencies.

Using sequence-based tests, they also showed low genetic differentiation between various field sites but strong associations between cytokine polymorphisms and levels of infection. *Il1b*, *Il2*, and *Il12* were associated with variation in a number of immunological parameters (e.g., IL-1b, IL-10, and IL-2 expression) and, in turn, with variation in resistance to multiple natural pathogens.

Paterson's results demonstrate that cytokines represent an important source of genetic variation for resistance to a range of pathogens in wild systems and that this genetic component was as important as sex and age in predicting infection status. He highlights the potential for future work to extend the range of genes studied—in tandem with further immunological assays—in order to understand how genetic diversity leads to functional variation in pathogen resistance in natural populations and, in turn, how such genetic diversity is maintained. In this respect, Paterson concluded by suggesting that wild immunology of rodents may be an informative model for the natural variation in immunity found in human populations.

### Autoimmunity and antibodies in the balance

Andrea Graham (assistant professor of ecology and evolutionary biology, Princeton University) spoke about using antibodies to understand immunoheterogeneity among the Soay sheep of St. Kilda in the Outer Hebrides. As suggested by Graham, antibodies are important immune effector molecules for a variety of infectious diseases, and they also present logistical advantages for studies of immunology in the wild. These advantages include relative stability *in vivo* and in the freezer; accurate measurement of antibodies requires small sample volumes (usually <5 $\mu$ l), which are more logistically feasible for many wild systems; and different antibody isotypes permit inference about the cytokine milieu in which a response is induced (e.g., Refs. 37 and 38), and, in principle, a wide array of antibody specificities—for example, to self versus non-self antigens, or across a range of parasite species and strains—are accessible for analysis. However, not all infections are cleared by antibodies and off-the-shelf reagents are not available for all host species, especially wild animals. Graham pointed out that investigators must therefore decide whether antibody measurements or other biomarkers are most suit-

able for the question and host–parasite system under study.<sup>9</sup>

The Soay sheep of St. Kilda present a wild system in which measurement of antibodies is both feasible and potentially informative. For example, thanks to the extensive demographic and genetic data collected during longitudinal study of the sheep,<sup>39</sup> antibody measurements can be analyzed in a whole-organism context, including assessment of associations between antibody titers and fitness.<sup>6</sup> The sheep are infected by and, in harsh winters with low food availability, are killed by intestinal nematodes.<sup>40</sup> Annual plasma samples are taken and veterinary immunological reagents that work in sheep are available.

The initial incentive for Andrea Graham, Dan Nussey, and colleagues to measure antibodies of the Soay sheep was to ask whether animals with limited food and abundant (possibly immunosuppressive) helminth infections exhibit autoimmunity. Thus, the first plasma molecules they chose to measure were antinuclear antibodies (ANA) that bind nuclear and cytoplasmic constituents of mammalian cells (for example, histones or tRNA). ANA are considered risk factors for systemic autoimmune diseases such as lupus.<sup>41</sup> However, ANA also include infection-protective natural antibodies<sup>42</sup> and/or may indicate highly responsive B cells.<sup>43</sup> Indeed, clinical evidence suggests that only after many years at high titers are ANA associated with disease.<sup>41</sup> It is currently unknown how wild animals balance effective parasite killing immune effector mechanisms, while limiting damage to self.

Graham's results suggest that despite food limitation and high parasite burdens, some Soay sheep were ANA<sup>+</sup> (based on clinical criteria). More interestingly, ewes with high ANA concentrations lived significantly longer than ewes with low ANA but reduced annual reproduction.<sup>6</sup> Due to these balancing associations, and possible trade-offs between survival and fecundity, the data suggest how immunoheterogeneity might be maintained in the wild and confirm a major prediction of life history theory.<sup>44</sup> However, any links between autoimmunity and resistance against infection (such as described by Ken Smith during the symposium; e.g., Ref. 45) in the Soay sheep are not yet clear. It is critical to forge such links, so Graham and colleagues are currently investigating relationships among titers of ANA, parasite-specific antibodies,

and natural antibodies—as well as the potential for maternal antibody transfer—to explain variation in survival among these sheep. Antibodies are protective against several developmental stages of the nematode *Teladorsagia circumcincta*,<sup>46</sup> which is among the most pathogenic parasites infecting the Soay sheep.<sup>40</sup>

The presentation highlighted that wild “model” systems, such as the Soay sheep of St. Kilda, provide fascinating new insights that are not provided by laboratory model systems. Graham concluded by suggesting that although the genetic and environmental variability of wild systems render detailed understanding of immune mechanisms difficult to obtain, they do permit analysis of how immune systems function, and why they vary, in the face of multiple natural challenges.

### Immunological evolution

Jim Kaufman (professor of comparative immunogenetics, University of Cambridge) discussed how studies of the immune response in birds and other non-mammalian vertebrates can tell us about pathogens, coevolution, and genomic organization. Kaufman highlighted that immune responses to most infectious or immunization challenges are enormously complex, presumably due to many millions of years of evolution that involved step-by-step acquisition of protective mechanisms adapted from different molecular and cellular processes, with each step responding to specific challenges. Kaufman suggested that while it is interesting to understand fundamental processes and principles underlying the evolution of immune responses, reconstructing the events that have occurred has been a difficult task. However, one component that is particularly amenable to such analysis is the adaptive immune system of vertebrates, which is clearly a *system* with a unique origin.<sup>47</sup> He referenced research on mammals by many groups over the last 40 years that have revealed key immune genes (such as genes encoding MHC, TcR, and antibodies), as well as the associated cells and processes that had already evolved at the emergence of the jawed vertebrates (see Ref. 47).

While many insights about the immune response have come from mammals, chickens have been a key organism for understanding the origin and evolution of the adaptive immune system.<sup>48</sup> Kaufman pointed out that advantages of the chicken, compared to non-mammalian vertebrates, are primar-

ily due to the economic and social importance of poultry as a food source. There is an enormous global poultry industry driving intense scrutiny of pathogens, genetics, and genomics, and immune responses to both diseases and vaccines. In addition, a wide range and huge number of chickens live in seminatural conditions and allow easy movement from field to lab and back again. Kaufman and colleagues have tried to understand why, in contrast to mammals, the chicken MHC has such strong genetic associations with resistance to infectious pathogens and responses to vaccines. They have found that chickens have single dominantly expressed class I and class II molecules, the properties of which can determine the immune response.<sup>49,50</sup> The basis for the dominantly expressed class I gene was due to coevolution with highly polymorphic TAP and tapasin genes located nearby.<sup>51,52</sup> In addition, surprisingly, NK receptor (NKR) genes are also present in the chicken MHC genomic region.<sup>52–54</sup>

The salient features that Kaufman and colleagues discovered are found in many, if not most, non-mammalian vertebrates, which suggests that the ancestral organization was like that of chickens, with the mammalian MHC arising by a “messy” inversion.<sup>47,48,55</sup> The presence of NKR genes suggests that ancestors of the receptors (such as NKR and T cell receptors) were also present in the primordial MHC, most likely in order to co-evolve with their ligands into a functioning system. Many other disparate data are explained by, and support, the view that the primordial MHC is the birthplace of the adaptive immune system.<sup>47,48,56</sup>

Chickens offer certain advantages to understanding the function and origin of the immune system over inbred laboratory mouse strains and outbred people. However, there are several issues for which wild animals, living without direct human intervention, would be more suitable. In particular, Kaufman suggests that the domestic chicken, even in the roughest barnyard setting, would not be an adequate system in which to understand immune responses of wild animals within the context of natural and complex ecosystems. Moreover, understanding the extent to which the salient features discovered in the chicken are general to other non-mammalian vertebrates can only be determined by studying many other species, most of which would be wild. Kaufman said that there is every reason to expect many exciting surprises in this quest but cautioned that

there are at least three groups working on the features of the MHC (biomedical scientists, farm animal health researchers, and evolutionary biologists), whose goals, methods, literatures, and citations are almost non-overlapping. In conclusion, Kaufman highlighted that increased dialogue between wild and lab studies can only improve matters, and provide novel insights into host–pathogen coevolution and the variations possible in the evolution of the immune response.

### The human dimension: polymorphism and autoimmunity

Ken Smith (professor of medicine, University of Cambridge) spoke about genetic predisposition to systemic lupus erythematosus (SLE), which is four to eight times more prevalent in people of African and Asian descent compared to people from European descent, and he presented evidence that this may, at least in part, result from selection for resistance to infection, in particular resistance to malaria. The risk of SLE is regulated by a large number of susceptibility alleles. A number of GWAS in thousands of lupus patients have identified over 35 loci that contribute to disease, but nonetheless explain only a relatively small proportion of the genetic contribution to SLE susceptibility.<sup>57</sup> One particularly important locus for SLE, implicated in both mouse and human, is on chromosome 1 and contains the low-affinity FcR (receptors that bind the Fc region of immunoglobulins) genes. The complexity of this region, due to it having arisen by duplication events and being prone to common copy number variation,<sup>58</sup> has resulted in it being largely excluded from SNP-based analyses.

Fc $\gamma$  receptors are important regulators of inflammatory responses to antigen–antibody immune complexes. They include both high- and low-affinity receptors, all of which have an activation function except for the inhibitory Fc $\gamma$ RIIB (CD32B), which is expressed by B cells, dendritic cells (DCs), macrophages, activated neutrophils, mast cells, and basophils.<sup>59</sup> Importantly, Fc $\gamma$ RIIB is the only Fc $\gamma$  receptor expressed on B cells in both humans and mice, and it controls many aspects of the antibody response, providing a form of feedback inhibition.

Fc $\gamma$ RIIB is found in an important but complex susceptibility locus for SLE in both mice and humans. Mice deficient in the receptor are prone to autoimmunity.<sup>59,60</sup> Consistent with this, mice over-

expressing Fc $\gamma$ RIIB are protected from autoimmunity;<sup>61</sup> Fc $\gamma$ RIIB also plays an important role in defense against bacterial infection.<sup>61,62</sup> Naturally occurring polymorphisms in the promoter region of Fc $\gamma$ RIIB have been found to be associated with reduced receptor expression and with autoimmune-prone strains,<sup>63,64</sup> but the variant most associated with SLE is very common in wild mice, suggesting an evolutionary advantage. This wild mouse variant has been “knocked-in” to a conventional mouse strain, indicating how subtle changes in Fc $\gamma$ RIIB regulation can drive autoimmunity (Espeli and Smith, in preparation). Human polymorphisms in Fc $\gamma$ RIIB include mutations in promoter regions that may control expression levels, but these have not been studied in detail.<sup>59</sup>

A further polymorphic allele in humans is a coding variant in the transmembrane domain (I232T). This allele, first associated with SLE in Japanese populations<sup>65</sup> (and subsequently shown to hold true in Europeans<sup>58</sup>), abolishes the inhibitory activity of Fc $\gamma$ RIIB.<sup>66,67</sup> Smith and others showed that this allele is homozygous in only around 1% of Europeans and between 5–10% of Africans and East Asians, ethnic groups known to suffer a higher incidence of SLE (at least when residing in the developed world). This association implies that an infectious agent might provide the balancing selection that would maintain the SLE-associated mutation at higher frequencies within some human populations.

This possibility was first tested in mice, and Fc $\gamma$ RIIB deficiency was found to confer resistance to *Plasmodium chabaudi* malaria through increased phagocytosis, inflammatory cytokine production, and antibody titers.<sup>45</sup> These results were later confirmed in experiments with lethal strains of rodent malaria.<sup>68</sup> Primary human phagocytes isolated from volunteers homozygous for the inactive I232T variant of Fc $\gamma$ RIIB showed heightened phagocytosis of *Plasmodium falciparum*-infected red blood cells,<sup>45</sup> which supports the hypothesis that a trade-off between resistance to malaria and propensity to develop SLE may be mediated by the Fc $\gamma$ RIIB locus.<sup>45</sup> To further test their hypothesis in the field, two cohorts of children with severe malaria from Kilifi in Kenya were genotyped, and homozygosity for T232 was associated with substantial protection against severe malaria, with an odds ratio of 0.56, similar to that conferred by heterozygous thalassaemia.<sup>69</sup>

These studies from Smith *et al.* are remarkable in that epidemiological observations made in humans were traced back, owing to their homologies in laboratory mice, to specific alleles that may explain the maintenance of autoimmune disease in humans as a consequence of selective pressures imposed by pathogens.

### Whipworm from the wild

Richard Grenis (professor of immunology, University of Manchester) spoke about the importance of the lab-generated mechanistic understanding of immunity to the whipworm, *Trichuris muris*. Specifically he highlighted the role of infective dose on the establishment of chronic infections and of the difficulty of deriving meaningful immunological readouts associated with patterns of infection in multiple infections. Whipworms are found in virtually every terrestrial mammal and are common in most wild rodents, reaching, for example, 88% prevalence in house mice. In 1954, *Trichuris muris* eggs were isolated from the feces of *Mus musculus* obtained from the birdhouse in the Zoological Park in Edinburgh.<sup>70</sup> Following embryonation, successful infection of laboratory mice was achieved, with low numbers of mature worms present in the caecum. Grenis pointed out that this strain of *T. muris* has subsequently been maintained successfully in laboratory mice in various laboratories around the world and has been used extensively as a system to study the host–parasite relationship with an emphasis on the immune response. The lab strain has helped generate the paradigm of Th2-mediated resistance to gastrointestinal helminths and dysregulation of this response leading to chronic infection.<sup>71</sup> In addition, Grenis highlighted that studies of this parasite strain have played a key role in identifying novel mechanisms of host protection and immunoregulation. Moreover, *T. muris* serves very well as a model of human Trichuriasis.

A small number of studies have explored *T. muris* infection in the wild and examined controlled infection in wild mice or outbred laboratory mice. In summary, data from field studies show that infection is generally characterized by low burdens of adult parasites (<20 worms), with only a few mice harboring many parasites. Grenis showed evidence that both laboratory-infected wild mice and outbred mice expelled their worm burden efficiently, although low-dose infections progressed readily to

patency. Further studies followed trickle infections in outbred mice in which repeated low-dose infections were given to mimic the manner in which animals in the field are likely to acquire infection. These studies demonstrated that low doses of eggs led to patent low-level infections, but for most mice, parasite numbers did not keep increasing, suggesting that some form of resistance against incoming parasites was operating.<sup>72,73</sup> Grenis suggested that the immunological basis of these observations only became apparent with the definition of resistance and susceptibility using inbred mice. Such studies defined key times post-infection that correlated with particular immune markers (such as cytokines, antibodies, and immunopathological features) and resistance or susceptibility to chronic infection. A series of experiments using low- or high-dose infections, given singularly or in multiples, including trickle infections, were subsequently carried out in inbred mice.<sup>74</sup> Grenis argued that the data clearly showed that when a single infection is given, assessment of immunological parameters is a very useful predictor of resistance status in terms of parasite burden. However, following complex infection regimes, such as trickle infection, the accuracy of these parameters as predictors of worm burden is markedly reduced, even in studies in which all other variables, such as sampling time, coinfection, and nutritional status, were carefully controlled. Nevertheless, in terms of worm burdens, there was evidence that some resistance could be built up over time in a manner similar to studies in wild mice.

Overall, the use of laboratory experiments with *T. muris* has been instrumental in defining mechanisms of resistance and susceptibility to intestinal nematode infection. Moreover, manipulation of infection burdens and regimes in inbred mice can reflect, at least in part, the complex situation that is experienced in the wild. Grenis finally drew attention to the fact that such studies do show, however, that inferring resistance status from analysis of immune parameters (known to play functional roles in well-defined conditions) remains a considerable challenge for the wild immunologist.

### Microbes in the picture

Markus Geuking (research fellow, University of Bern) in collaboration with Kathy McCoy (professor, University of Bern) presented their work on the maintenance of intestinal homeostasis in response

to controlled colonization with commensal bacteria. Geuking began his presentation by reminding the audience that humans are born germ free and then subsequently colonized with commensal bacteria. The highest density of bacteria ( $>10^{11}$  CFU/gram) is found in the colon, and these bacteria are kept from entering the host by a single layer of epithelial cells covered by mucus—as the only physical barrier. Interestingly, humans have over 50% of all immune cells located in the intestine. Geuking asks how the relatively peaceful coexistence of bacteria and immune cells is maintained.

Vertebrates have coevolved with the community of intestinal microbes (microbiota), and they usually coexist in peaceful mutualism. Although these bacteria carry powerful inflammatory patterns that can be recognized by Toll-like receptors and other pattern recognition receptors, the intestinal immune system has adapted to the presence of these bacteria and does not, in healthy individuals, induce an inflammatory response. To study the mechanisms of intestinal immune adaptation to the presence of commensal bacteria, Geuking and colleagues use axenic (germ-free) and gnotobiotic (defined microbiota) mouse models.<sup>75</sup> This allows them to control and define the microbiota present, as well as to manipulate the host immune system by using genetically modified mouse strains that are re-derived to germ-free status by axenic two-cell embryo transfer before being colonizing with a defined microbiota.

Several studies have investigated the immune responses to individual bacterial species.<sup>76–79</sup> To study the adaptation of the intestinal CD4<sup>+</sup> T cell compartment to colonization with a truly mutualistic commensal microbiota, Geuking *et al.* colonized germ-free mice with the defined altered Schaedler flora, which consists of eight species. They found that commensal colonization induced a strictly compartmentalized T<sub>reg</sub> cell response in the colon lamina propria.<sup>80</sup> This T<sub>reg</sub> response was functional because the same benign microbiota induced Th1 and Th17 effector responses in a mouse strain with defective T<sub>reg</sub> cells (due to transgenic expression of a virus-specific T cell receptor), which were controlled by transferred wild-type T<sub>reg</sub> cells.

Geuking concluded his talk by underlining the importance of immunological adaptations to the microbiota in maintaining gut homeostasis. He suggested that given that colonization of “true” commensals induces a regulatory T cell response that

controls proper adaptation of the intestinal CD4<sup>+</sup> T cell compartment, it is likely that more complex microbiota that also harbor potential opportunistic bacteria and parasites, as seen in wild hosts, may determine the ability of hosts to generate immune responses against pathogens.

## A wild world within

Peter Turnbaugh (Bauer Fellow, Harvard University) presented recent research exploring the differences and similarities of the gut microbiomes of laboratory and wild mammals. Humans and other mammals have coevolved with trillions of microorganisms, whose aggregate genomes often contribute functions not encoded by our own human genes.<sup>81</sup> The largest collection of these microorganisms, whose genomes are together referred to as the gut microbiome, is found throughout the gastrointestinal tract and can have a profound influence on health and disease, such as affecting nutrition and energy balance (i.e., diabetes, obesity, and metabolic syndrome, e.g., Refs. 82 and 83), inflammatory bowel disease,<sup>84,85</sup> immune development,<sup>78</sup> cardiovascular disease,<sup>86</sup> and xenobiotic metabolism.<sup>87,88</sup> However, unlike the human genome, the gut microbiome can be shaped by a variety of environmental factors, such as drug use,<sup>89</sup> diet,<sup>90</sup> probiotics,<sup>91</sup> and delivery method.<sup>92</sup> Turnbaugh highlighted that this plasticity, coupled with physiological relevance, makes the gut microbiome an attractive target for personalized medicine and/or nutrition.

To date, many of the studies of the mammalian gut microbiome have focused on inbred mouse lines in controlled laboratory settings. For example, comparisons of obese and lean mice have demonstrated differences in their gut microbiomes, including an increased relative abundance of genes for dietary energy harvest.<sup>93,94</sup> Interestingly, these “obesity-associated” communities can be used to transmit host adiposity: recipient germ-free mice colonized with a sample taken from an obese donor have a significantly greater gain in adiposity than mice colonized from a lean donor.<sup>93,94</sup> In an attempt to bridge the gap between laboratory mice and humans, Turnbaugh and colleagues have recently turned to “humanized mice”: formerly germ-free animals colonized with a human donor sample.<sup>90</sup> These animals are then used to investigate the distribution of bacteria throughout the length of their gastrointestinal tract and the succession of the

gut microbial community early in life. Comparisons of humanized mice fed a low-fat polysaccharide-rich chow diet or a high-fat/high-sugar “Western” diet emphasize that dietary shifts can have a consistent, rapid, and significant impact on microbial community structure, gene content, and gene expression.<sup>90</sup>

Turnbaugh highlighted the fact that humans, the most commonly studied “wild animal,” show similar shifts in microbial community structure and gene content when comparing obese and lean individuals,<sup>95,96</sup> although recent studies suggest that this relationship may be complicated by a variety of host and environmental factors.<sup>97</sup> Of note, a large-scale analysis recently identified three functional modules—including ATPases—in the gut microbiome that were correlated with body mass index.<sup>98</sup> Furthermore, recent studies have emphasized the dependence of the human gut microbial community on diet.<sup>99–101</sup>

Humans appear to be representative of other omnivorous mammals. Turnbaugh described a recent study of humans and 59 other mammalian species from two zoos and the wild. The study results emphasized the broad similarities among the gut microbiota of species spanning the mammalian phylogeny, gut physiologies, and diets.<sup>102</sup> Microbial communities did not group according to habitat (wild versus captive). However, the samples did group based on diet (carnivorous, omnivorous, or herbivorous), with the notable exception of pandas and bears that grouped according to host phylogeny. Interestingly, an analysis of the gene content of these communities revealed that the representation of functional groups of genes correlates with community structure (measured by the 16S rRNA gene sequence) and that carnivore and herbivore microbiota have a greater relative abundance of genes for amino acid catabolism and biosynthesis, respectively.<sup>103</sup>

A major challenge moving forward, Turnbaugh suggested, will be to interface with other fields of study relevant to the gut microbiomes of humans and other mammals. Specifically, what lessons from macro-ecology, wild animal ecology, immunology, and/or parasitology can be applied to studies of the gut microbiome? Can detailed surveys of wild animal populations be used to interpret and guide ongoing studies of the human microbiome? Are the gut microbiomes of laboratory mice representative

of their natural colonization, or have they diverged from their wild counterparts? What role do parasites or other small eukaryotes play in shaping the bacterial community and its interactions with the host? And what role does host biogeography play in shaping microbial communities? Together, Turnbaugh concluded, these studies stand to address fundamental biological questions, while also leading toward a promising future of microbiome-based therapies and diagnostics.

## Conclusion

The wild immunology symposium concluded with a wide-ranging discussion of the lessons from wild immunology and the next steps for the field. Many of the conclusions immunologists have drawn from the clinic and the laboratory have been reassuringly validated by studies on wild populations, in particular the link between immunological polymorphisms and infectious agents, providing further elegant examples of how pathogens may be maintaining genetic variants in many different host species. The striking parallels between autoimmune propensities in humans, mice, and sheep give further credence to the idea that studies on free-living nonhumans can provide not only a test bed, but a mirror to reveal the relative significance of the many parameters that are thought to regulate optimal immune function. In addition, laboratory-based immunology provides important mechanistic insight that can be incorporated into wild studies and results that need to be tested in natural systems, where their relevance to fitness and health can be investigated. Research in wild immunology will become increasingly valuable as we further investigate the key factors in genetics, ecology, and infection that influence immune status in the real world.

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## Conflicts of interest

The authors declare no conflicts of interest.

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