Figure 2. Submissions per year (2006–2012).

How do we evaluate how well the *Journal* is succeeding? By utilizing objective metrics to assess impact, reputation, and value to the scientific community. The American Thoracic Society journals were recently reviewed by an advisory committee, who positively evaluated our performance and provided specific recommendations to promote future growth and improvements. Figure 3 presents the data on downloads of content from the *AJRCCM* website in the past several years. This steady increase reflects the growing impact of the *Journal* and the way it is accessed. This also reflects the fact that the *Journal* is read broadly and is relevant to a great number of clinicians and scientists in our field.

We are rightfully proud of the position of the *AJRCCM* as the premier journal in our specialty. We look forward to continuing its improvement through new initiatives from our Editors, ATS-sponsored workshops, external advisors, and the ATS leadership. We must continue to adapt as new technological innovations present novel opportunities to disseminate and publish the best science in our field. We also look to you, our readers, for feedback on

Figure 3. Full-text HTML and PDF downloads (2009–2012).

how the *Journal* can meet and anticipate your needs. Thank you for giving us the opportunity to be of service to you.

Author disclosures are available with the text of this article at www.atsjournals.org.

JACOB I. SZNAJDER, M.D.

LAURENT BROCHARD, M.D.

G. R. SCOTT BUDINGER, M.D.

SERPIL C. ERZURUM, M.D.

ELLIOT ISRAEL, M.D.

PAUL SCHUMACKER, PH.D.

American Journal of Respiratory and Critical Care Medicine

Copyright © 2013 by the American Thoracic Society

DOI: 10.1164/rccm.201303-0409ED

Humanizing the Mouse: In Defense of Murine Models of Critical Illness

Murine models have been used to understand the mechanisms of inflammatory disease for decades and have become an indispensable tool for the vast majority of laboratories across the globe. Several key advantages of murine models explain their wide adoption by the scientific community. Most importantly, the discovery that embryonic stem cells from mice could be genetically manipulated to create transgenic or knockout animals allowed investigators to establish causal links between genes and disease phenotypes and earned Drs. Mario R. Capecchi, Martin J. Evans, and Oliver Smithies the Nobel Prize in Physiology and Medicine in 2007. The subsequent development of Cre-lox and other recombinant DNA technologies allowed examination of the tissue-specific role played by genes in the disease pathogenesis and revolutionized our understanding of normal development. These genetically engineered mice have provided valuable insights into the development and progression of numerous diseases and have facilitated the development of novel diagnostic and therapeutic approaches for the treatment of human

disease. The laboratory mouse offers investigators several additional advantages including the provision of several well-characterized and easily available inbred genetic strains, a host of laboratory reagents available for detailed phenotypic analysis, a wealth of genetic and transcriptional expression databases, relatively short breeding times, and low housing costs.

Despite these clear advantages, there exists ongoing concern that findings in mice might have little relevance for human immune-mediated disease (1–6). These concerns arise from recognized differences between the murine and human immune system that might affect both innate and adaptive immunity (7). In addition, the uniform genetic background in murine models, which is often cited as an advantage, can be seen as a disadvantage given the disparate genetic backgrounds characteristic of human disease. In a recent study by the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program, the authors attempted to examine differences or similarities between murine models and humans in common clinical problems that result in critical illness, specifically endotoxemia, burns, and trauma (8). The authors performed transcriptional analysis of RNA isolated from pooled peripheral blood leukocytes obtained from patients at different phases of their critical illness with what they estimated to be the corresponding times in the evolution of the murine models. They found that, while

Supported by grants from NIH or the VA BX000201, ES013995, HL071643, and HL071643 (to G.R.S.B.); GM029507 and GM061656 (to P.A.W.); and AR050250, AR054796, AI092490, and HL108795 as well as funds provided by Solovy/Arthritis Research Society Professor (to H.P.).

there was a strong correlation between the pooled leukocyte transcriptome in patients with burns, trauma, and endotoxemia, these transcriptional responses correlated poorly with the murine models. Furthermore, there was little correlation between the transcriptional profiles in mice in the three different models or in the transcriptional response of mice and humans following the intravenous administration of low-dose LPS. The large amount of attention engendered by this report justifies a more detailed consideration of the design and interpretation of this study (9).

The investigators analyzed RNA from pooled leukocytes obtained from peripheral blood. This choice was likely dictated by the inability to obtain other tissues from this fragile, critically ill patient population. Nevertheless, the circulating leukocyte pool in humans (predominantly neutrophils with a paucity of lymphocytes) is markedly different from that of mice (predominantly lymphocytes with a paucity of neutrophils). It is therefore somewhat unsurprising that the transcriptional profile of pooled leukocytes would differ in mice and humans in any disease model. One wonders whether the transcriptional profile of immune cell populations in the organs primarily affected by the disease might have been more similar (i.e., a comparison of the transcriptional profile of cells in the burned skin or the region of trauma). Some evidence suggests this might be the case; for example, Yu and coworkers compared the transcriptional profile of lung homogenates in a common murine model of asthma with endobronchial biopsies from patients with asthma. They found a high degree of correlation between the transcriptional signatures in the two samples, and they were able to use this analysis to highlight the importance of mast cells in this response (10). In addition to this concern, the assumption that circulating immune cells are representative of the immune response in the affected organs requires cautious interpretation. Indeed, the finding that distinct disorders, which differ except in their requirement for intensive medical care, share such a similar transcriptional profile in the circulating leukocyte pool, suggest that these changes may be secondary to the critical illness rather than causally related to the disease pathogenesis. These similarities might reflect the similar treatment they received after admission to the intensive care unit, which likely included numerous medical therapies known to affect immune function, including antibiotics, adrenergic agonists, steroids, and fluid resuscitation, among others. In contrast, the mice were left untreated in all of the murine models.

Several methodological concerns limit the conclusions of the article. While the investigators attempted to “match” the time course of the murine models with the human disease, this estimation is necessarily imprecise, particularly as murine models are designed to evolve over a shorter time course (and are therefore often more severe) than the illness they model (11). Another issue is that none of the genetic changes identified in the microarray datasets were validated using standard PCR techniques. Moreover, the authors limited their analysis to human genes with identified murine orthologs, even though it is clear that multiple genes and proteins that are present in humans do not have direct homologs in mice and *vice versa* (12). For example, the investigators found that expression of the human gene (HLA-DR) changes significantly in human critical illness while its murine ortholog (H2-EA-PS) does not. They use this example to illustrate the differences in disease evolution in human and murine models; however, this interpretation is flawed, as this murine gene is a pseudogene. Finally, while the study was powered to identify strong similarities, the small number of mice does not provide sufficient power to conclude that there are no similarities.

In light of these important concerns, the investigators’ suggestion that murine models may mislead the development of therapies for human disease appears poorly supported. While the authors cite the failure of anti-cytokine data to improve outcomes in sepsis as an example, it seems more likely that this failure resulted from an underappreciation of the importance of the innate immune system in effective pathogen clearance, as well as the substantial overlapping functions of chemokines and cytokines. This hypothesis is supported by the observation that the early administration of antibiotics remains one of the few therapies clearly associated with improved outcomes in patients with sepsis. Perhaps a careful search of this dataset looking for changes that occurred in both mice and humans would identify novel transcriptionally mediated pathways that play a role in the pathogenesis of human critical illness.

Despite the problems with this particular report, recognized differences between the murine and human immune systems beg for improved model systems. One solution is represented by the successful engraftment of a human immune system into mice, creating the “humanized mouse.” Initial attempts to engraft human immune cells into various immunodeficient mice led to poor and short-term engraftment resulting from a persistent murine immune response to the human xenografts. A new generation of immunodeficient murine strains has dramatically altered this landscape (BALB/c-*Rag2*^{-/-} *IL2r*^{-/-}, NOD-*Rag1*^{-/-} *IL2r*^{-/-}, and NOD-*scid* *IL2r*^{null}) (13). While the complete loss of mature T and B cells in *Rag1/2*-deficient or *Scid* mice allowed for partial engraftment of human hematopoietic stem cells, dramatic improvements in engraftment were only observed with concomitant inactivation of the γ chain of the IL-2 receptor. This receptor contributes to the innate immune response via signaling through several interleukins and the development of natural killer (NK) cells (13, 14). These mice support long-term engraftment of human hematopoietic stem cells, which undergo multilineage development, resulting in a fully functional human immune system, including T, B, NK, and dendritic cells, as well as monocytes/macrophages and granulocytes. The model has been further improved by genetic modifications of the transplanted human stem cells (e.g., transfection of CD47) and the murine host (e.g., expression of human M-CSF, HLA molecules, thrombopoietin, IL-3, SCF, or GM-CSF and deletion of the mouse MHC class I and II alleles) (15). The humanized mouse model has already revolutionized studies of infectious diseases that specifically target human hosts including HIV (14, 16), Dengue virus (17, 18), and *Salmonella typhi* (19–21). A few studies have used this model to examine autoimmune diseases such as type 1 diabetes (22, 23), lupus (24), and inflammatory arthritis (25, 26), and recent reports have examined the response of humanized mice to sepsis (27, 28).

In summary, the data from the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program highlight important concerns about translating findings in murine models of sepsis and other immune mediated diseases to humans. However, the lack of correlation between peripheral blood transcriptional profiles in mice and humans should not be used to argue against the utility of murine models. This conclusion is not supported by the data and fails to recognize the history of discovery to which murine models have contributed. In addition, this conclusion leaves us with little room for hope as detailed mechanistic studies in humans with critical illness are unlikely to be technically, financially, or ethically feasible. Instead, results like these should be interpreted as a research challenge that requires innovative solutions. Humanized mice represent one strategy, but others, including the generation of induced pluripotent stem cells from patients with disease, are under development. All of these efforts will require the continued careful work

of basic and clinician-scientists and strong support from the NIH and other funding agencies.

Author disclosures are available with the text of this article at www.atsjournals.org.

HARRIS PERLMAN, PH.D.
G. R. SCOTT BUDINGER, M.D.
*Feinberg School of Medicine
Northwestern University
Chicago, Illinois*

PETER A. WARD, M.D.
*Department of Pathology
University of Michigan Medical School
Ann Arbor, Michigan*

References

- Rivera J, Tessarollo L. Genetic background and the dilemma of translating mouse studies to humans. *Immunity* 2008;28:1–4.
- Rämer PC, Chijioke O, Meixlsperger S, Leung CS, Münz C. Mice with human immune system components as in vivo models for infections with human pathogens. *Immunol Cell Biol* 2011;89:408–416.
- Khanna R, Burrows SR. Human immunology: a case for the ascent of non-furry immunology. *Immunol Cell Biol* 2011;89:330–331.
- Davis MM. A prescription for human immunology. *Immunity* 2008;29:835–838.
- Schneemann M, Schoeden G. Macrophage biology and immunology: man is not a mouse. *J Leukoc Biol* 2007;81:579–580, discussion 580.
- Sabroe I, Dockrell DH, Vogel SN, Renshaw SA, Whyte MK, Dower SK. Identifying and hurdling obstacles to translational research. *Nat Rev Immunol* 2007;7:77–82.
- Legrand N, Weijer K, Spits H. Experimental models to study development and function of the human immune system *in vivo*. *J Immunol* 2006;176:2053–2058.
- Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, *et al.*; Inflammation and Host Response to Injury, Large Scale Collaborative Research Program. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 2013;110:3507–3512.
- Kolata G. Mice fall short as test subjects for humans' deadly ills. *New York Times*. 2013 February 11 [accessed 2013 Feb 11]. Available from: http://www.nytimes.com/2013/02/12/science/testing-of-some-deadly-diseases-on-mice-mislead-report-says.html?_r=0
- Yu M, Eckart MR, Morgan AA, Mukai K, Butte AJ, Tsai M, Galli SJ. Identification of an IFN- γ /mast cell axis in a mouse model of chronic asthma. *J Clin Invest* 2011;121:3133–3143.
- Eichacker PQ, Parent C, Kalil A, Esposito C, Cui X, Banks SM, Gerstenberger EP, Fitz Y, Danner RL, Natanson C. Risk and the efficacy of anti-inflammatory agents: retrospective and confirmatory studies of sepsis. *Am J Respir Crit Care Med* 2002;166:1197–1205.
- Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004;172:2731–2738.
- Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007;7:118–130.
- Van Duyne R, Pedati C, Guendel I, Carpio L, Kehn-Hall K, Saifuddin M, Kashanchi F. The utilization of humanized mouse models for the study of human retroviral infections. *Retrovirology* 2009;6:76.
- Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. *Nat Rev Immunol* 2012;12:786–798.
- Legrand N, Ploss A, Balling R, Becker PD, Borsotti C, Brezillon N, Debarry J, de Jong Y, Deng H, Di Santo JP, *et al.* Humanized mice for modeling human infectious disease: challenges, progress, and outlook. *Cell Host Microbe* 2009;6:5–9.
- Bente DA, Melkus MW, Garcia JV, Rico-Hesse R. Dengue fever in humanized NOD/SCID mice. *J Virol* 2005;79:13797–13799.
- Jaiswal S, Pearson T, Friberg H, Shultz LD, Greiner DL, Rothman AL, Mathew A. Dengue virus infection and virus-specific HLA-A2 restricted immune responses in humanized NOD-scid IL2rgammanull mice. *PLoS ONE* 2009;4:e7251.
- Libby SJ, Brehm MA, Greiner DL, Shultz LD, McClelland M, Smith KD, Cookson BT, Karlinsey JE, Kinkel TL, Porwollik S, *et al.* Humanized nonobese diabetic-scid IL2rgammanull mice are susceptible to lethal *Salmonella typhi* infection. *Proc Natl Acad Sci USA* 2010;107:15589–15594.
- Hatzioannou T, Evans DT. Animal models for HIV/AIDS research. *Nat Rev Microbiol* 2012;10:852–867.
- Akkina R. New generation humanized mice for virus research: comparative aspects and future prospects. *Virology* 2013;435:14–28.
- Whitfield-Larry F, Young EF, Talmage G, Fudge E, Azam A, Patel S, Largay J, Byrd W, Buse J, Calikoglu AS, *et al.* HLA-A2-matched peripheral blood mononuclear cells from type 1 diabetic patients, but not nondiabetic donors, transfer insulinitis to NOD-scid/ γ c(null)/HLA-A2 transgenic mice concurrent with the expansion of islet-specific CD8 $^{+}$ T cells. *Diabetes* 2011;60:1726–1733.
- Brehm MA, Bortell R, Diiorio P, Leif J, Laning J, Cuthbert A, Yang C, Herlihy M, Burzenski L, Gott B, *et al.* Human immune system development and rejection of human islet allografts in spontaneously diabetic NOD-Rag1null IL2rgammanull Ins2Akita mice. *Diabetes* 2010;59:2265–2270.
- Andrade D, Redecha PB, Vukelic M, Qing X, Perino G, Salmon JE, Koo GC. Engraftment of PBMC from SLE and APS donors into BALB-Rag2 $^{-/-}$ IL2Rgc $^{-/-}$ mice: a promising model for studying human disease. *Arthritis Rheum* 2011;63:2764–2773.
- Kuwana Y, Takei M, Yajima M, Imadome K, Inomata H, Shiozaki M, Ikumi N, Nozaki T, Shiraiwa H, Kitamura N, *et al.* Epstein-Barr virus induces erosive arthritis in humanized mice. *PLoS ONE* 2011;6:e26630.
- Misharin AV, Haines GK III, Rose S, Gierut AK, Hotchkiss RS, Perlman H. Development of a new humanized mouse model to study acute inflammatory arthritis. *J Transl Med* 2012;10:190.
- Ye C, Choi JG, Abraham S, Wu H, Diaz D, Terreros D, Shankar P, Manjunath N. Human macrophage and dendritic cell-specific silencing of high-mobility group protein B1 ameliorates sepsis in a humanized mouse model. *Proc Natl Acad Sci USA* 2012;109:21052–21057.
- Ernst W, Zimara N, Hanses F, Männel DN, Seelbach-Göbel B, Wege AK. Humanized mice, a new model to study the influence of drug treatment on neonatal sepsis. *Infect Immun* 2013;81:1520–1531.

Copyright © 2013 by the American Thoracic Society
DOI: 10.1164/rccm.201303-0489ED

Chronic Obstructive Pulmonary Disease Genomics: Yesterday, Discovering Population Biomarkers; Tomorrow, Defining Disease Clusters

The use of transcriptomics for gene discovery in lung diseases, and chronic obstructive pulmonary disease (COPD) in general, has generally followed similar studies of diseases of other organs, particularly cancer. Likewise, limitations of transcriptomics studies of COPD have mirrored those of other diseases, mainly being the failure to confirm or validate major observations. This is

often ascribed to variability between cohorts and individuals, or between study-specific experimental designs and analytical approaches. Regardless of the cause, these problems severely limit the impact and potential utility of the reported results.

The first reported genome-wide study of gene expression in COPD was completed by Zhang and coworkers in 2000 (1).