Moving Forward: Recent Developments for the Ferret Biomedical Research Model

Randy A. Albrecht, Wen-Chun Liu, Andrea J. Sant, S. Mark Tompkins, Andrew Pekosz, Victoria Meliopoulos, Sean Cherry, Paul G. Thomas, Stacey Schultz-Cherry

Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA
David H. Smith Center for Vaccine Biology and Immunology, Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, USA
Center for Vaccines and Immunology, University of Georgia, Athens, Georgia, USA
W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA
Department of Immunology, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA
Department of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA

ABSTRACT

Since the initial report in 1911, the domestic ferret has become an invaluable biomedical research model. While widely recognized for its utility in influenza virus research, ferrets are used for a variety of infectious and noninfectious disease models due to the anatomical, metabolic, and physiological features they share with humans and their susceptibility to many human pathogens. However, there are limitations to the model that must be overcome for maximal utility for the scientific community. Here, we describe important recent advances that will accelerate biomedical research with this animal model.

KEYWORDS

advances, animal model, ferret

In 1911, the first study using the domestic ferret, Mustela putorius furo, for biomedical research was published (1). Since then, the ferret has been an invaluable model for cardiac research (2), spinal cord injury (3), epilepsy (4), and several lung conditions, including smoke-induced chronic obstructive pulmonary disease (COPD) (5), cystic fibrosis (6), and tobacco-induced lung cancer (7). The recent development of a database of the anatomical connections and structural features of the ferret brain will likely also improve the relevance of this model for neurological research (8). Yet, the ferret model is most widely recognized for its utility in infectious disease research, especially respiratory infections (Table 1). A variety of human pathogens are known to naturally infect ferrets and often reproduce human disease better than mouse models. In this article, we discuss the recent advances and ongoing initiatives to increase the utility of the ferret model for biomedical research.

MODELS, GENOMES, AND OMICS

The first transgenic ferret was produced by somatic cell nuclear transfer (SCNT) to oocyte recipient cells in 2006 (9). This technique was then combined with adeno-associated virus-mediated gene targeting of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to generate a transgenic ferret model of cystic fibrosis and create the first reported ferret genomic bacterial artificial chromosome library (10). More recently, CRISPR/Cas9-mediated genome editing techniques were applied to ferrets to develop a model organism to study X-linked, double cortin-related lissencephaly spectrum (11). In addition to genetically modified ferrets, research groups have described the development of immunocompromised (12), pregnant (13), aged (14), and diet-induced obese (DIO [unpublished data]) models to understand disease in high-risk
populations. It is likely that new models and transgenic animals will be developed in the near future.

The sequencing of the ferret genome (15) was instrumental in advancing functional genomic analysis. Numerous groups developed reagents to monitor gene-specific mRNA expression levels via TaqMan-based or Sybr green-based real-time reverse transcription-PCR assays for a plethora of targets. Many of these primers are available free of charge through the National Institute of Allergy and Infectious Diseases (NIAID) established BEI Resources (https://www.beiresources.org/Home.aspx). Bruder et al. described the development of an expression microarray platform that included the identification of 41 genes with consistent baseline transcription profiles across tissues that could be used as housekeeping genes (16). Our group developed and is validating a FLUIDIGM panel with 144 distinct immune response and lung injury and repair genes. Beyond transcription, Tisoncik-Go et al. described an integrated omics analysis that profiles lipids, metabolites, and proteins in the respiratory compartments of influenza virus-infected ferrets (17). Combined, these tools provide powerful resources to the research community.

THE NEXT FRONTIER: THE IMMUNE RESPONSE

Despite its relevance for biomedical research, there are limitations of the ferret model for immunologic studies due to the dearth of reagents. Screening of commercially available antibodies for cross-reactivity with markers on innate and adaptive cell subsets and cytokines in ferrets has yielded limited success (Table 2). To resolve this, a group of researchers from around the world are working together to develop validated reagents and assays to improve our understanding of the innate and adaptive immune responses in the ferret.

To date, recombinant proteins representing a range of intrinsic, innate, and adaptive immune markers are under development, and some are already available from commercial sources (18, 19). These include type I and III interferons (IFNs), RIG-I and Toll-like receptors, cytokines, and chemokines, as well as cell surface markers for immune and nonimmune cells. In terms of adaptive immune responses, Kirchenbaum and Ross recently developed a monoclonal antibody against the ferret B cell receptor light chain that is useful in distinguishing kappa versus lambda B cell responses (20, 21). Enzyme-linked immunosorbent spot (ELISpot) and flow cytometric assays have been developed to quantify the isotypes of antibody-secreting cells (IgG or IgA) (22), pan-B cells (CD20⁺, CD79a⁺), and Ig⁺ B cells (18, 19). T cell phenotyping has been limited to quantification of overall CD3⁺ T cells, including CD4⁺ and CD8⁺ subsets, by flow cytometric assays.

### TABLE 1 Human microbes used in the ferret model

| Pathogen group and species                        | Reference(s) |
|--------------------------------------------------|--------------|
| **Viruses**                                      |              |
| Influenza virus                                  | 26           |
| Respiratory syncytial virus                      | 27, 28       |
| *Metapneumovirus*                                | 29           |
| Measles virus                                    | 30           |
| Mumps virus                                      | 31, 32       |
| Parainfluenza viruses                            | 33, 34       |
| Severe acute respiratory syndrome coronavirus    | 35           |
| Nipah virus                                      | 36           |
| Ebola virus                                      | 37           |
| Rift Valley fever virus                          | 38           |
| **Bacteria**                                     |              |
| Streptococcus spp.                               | 39           |
| *Staphylococcus aureus*                          | 40           |
| *Helicobacter mustelae*                          | 41           |
| *Mycobacterium*                                  | 42           |
| **Fungi**                                        |              |
| *Pneumocystis jirovecii*                         | 43           |

mbio.asm.org
and identification of antigen-specific effector responses by detecting IFN-γ secretion in flow-based intracellular cytokine secretion assays or ELISpot assays (18). An in vivo depletion of CD8 T cells using a cross-reactive human monoclonal antibody has been shown to delay influenza virus clearance (23). To increase our toolbox, the Centers for Excellence in Influenza Research and Surveillance (CEIRS) network has undertaken a large project to rapidly produce monoclonal antibodies and develop assays to support the universal influenza vaccine initiative (24). Antibodies in production include B cell markers (CD83, CD86, CD95, CD19, CD20, CD25, CD27, CD38, CD138, CXCR5, and CCR) and T cell markers (CD4, CCR7, CD3e, CD40, CD40L, CD44, CD62L, CD69, CD103, PD-1, CXCR3, interleukin-7 receptor [IL-7R], and IL-15Ra) and others (CXCR4, CD140, IL-2, IL-21, and IL-4). These much-needed reagents will facilitate efforts to establish immunologic assays to interrogate the innate and adaptive immune responses to infection and vaccination at the level of detail that is routinely applied to studies of mouse or human immunology. Importantly, the ferret model will allow correlates of protection to be established after vaccination and infection in conjunction with transmission studies, which are not available in the mouse models. Additionally, the longer life span of the ferret relative to the mouse will allow analysis of the evolution of the immune response to sequential infection and/or vaccination (25), permitting more accurate modeling of the immune response in humans.

### WAYS FORWARD

While there has been exciting progress, much work remains to move the ferret model forward. Toward this goal, the CEIRS group has produced fibroblasts and primary nasal and tracheal epithelial cells and cell lines, established a repository of defined tissues and cell types (Table 3), and are working with the J. Craig Venter Institute to define the ferret major histocompatibility complex (MHC). An exciting achievement is the completion of the PacBio sequencing of the ferret MHC (Granger Sutton, personal communication). While these are important steps, the ultimate goal is to provide the

---

**TABLE 2 Commercial kits and immunologic reagents tested in the ferret model**

| Product type and name | Specificity | Clone | Isotype | Host | Vendor | Application | Reference(s) |
|-----------------------|-------------|-------|---------|------|--------|-------------|--------------|
| Commercial kits       |             |       |         |      |        |             |              |
| LIVE/ DEAD Fixable Aqua dead cell stain | Mouse | IM7 | IgG2b, k | Rat | BD Pharmingen | Flow cyt | 18 |
| IFN-γ ELISpot basic (HRP) kit | Mouse | MabTech | ELISpot | 18 |
| Primary antibodies    |             |       |         |      |        |             |              |
| CD4                   | Mouse/human | M1/70 | IgG2b, k | Rat | BD Pharmingen or BioLegend | Flow cyt | 18, 19 |
| CD8a                  | Human       | OK-T8 | IgG2a   | Mouse | eBioscience/Tonbo | Flow cyt | 18, 19 |
| CD4                   | Ferret      | 02    | IgG1    | Mouse | Sino Biological | Flow cyt | 18, 19 |
| MHC-II                | Human       | L243  | IgG2a, k | Mouse | BioLegend | Flow cyt | 18 |
| IgA, IgM, IgG         | Ferret      | Poly | Goat    | LSBio | Flow cyt | 18 |
| CD59                  | Mouse       | AL-21 | IgM, k  | Rat | BD Pharmingen | Flow cyt | 18 |
| CD79a                 | Human       | HM47  | IgG1, k | Mouse | eBioscience | Flow cyt | 18 |
| CD20                  | Ferret      | 71    | IgG     | Rabbit | Sino Biological | Flow cyt | 18 |
| CD3                   | Human       | SS033 | Poly | Rabbit | Dako | IHC | 44 |
| Lysozyme              | Human       | A0099 | Poly | Rabbit | Dako | IHC | 44 |
| CD20                  | Human       | RB-901-P | Poly | Rabbit | Thermo (Fisher) | IHC | 44 |
| CD79a                 | Human       | HM57  | IgG1, k | Mouse | Dako | IHC | 44 |
| MHC-II                | Human       | TAL 185 | IgG1, k | Mouse | Dako | IHC | 44 |
| CD3                   | Human       | PC3/188A | IgG1, k | Mouse | Santa Cruz Biotech | Flow cyt | 45 |
| IFN-γ (capture Ab)    | Cow         | CC302 | IgG1   | Mouse | Bio-Rad | ELISpot/Flow cyt | 45 |
| IFN-γ biotinylated (detection Ab) | Dog | Poly | Goat | R&D Systems | 45, 46 |

*aAbbreviations: HRP, horseradish peroxidase conjugate; TNF, tumor necrosis factor; Ab, antibody; Flow cyt, flow cytometry; IHC, immunohistochemistry.*
biomedical research community with validated reagents and protocols they can trust to ensure the rigor and reproducibility in experiments utilizing the ferret model. In support of this goal, many of the reagents created through the CEIRS network will be made publicly available through the CEIRS Data Processing and Coordinating Center (DPCC) website (http://www.niaidceirs.org/resources/ceirs-reagents/).

ACKNOWLEDGMENTS

We thank everyone involved in Team Ferret, whose names we will not list for fear we might miss someone, as well as others producing reagents for the ferret model. We also thank Diane Post (NIAID) and the members of the CEIRS network for feedback, advice, and constructive criticism.

Finally, our funding sources included HHSN272201400006C (St. Jude’s CEIRS), HHSN272201400008C (CRIP CEIRS), HHSN272201400007C (Johns Hopkins CEIRS), HHSN272201400004C (Emory-UGA CEIRS), and HHSN272201400005C (NYICE).

REFERENCES

1. Yeates T. 1911. Studies in the embryology of the ferret. J Anat Physiol 45:319–335.
2. Truex RC, Belez R, Ginsberg LM, Hartman RL. 1974. Anatomy of the ferret heart: an animal model for cardiac research. Anat Rec 179:411–422. https://doi.org/10.1002/ar.1091790402.
3. Eidelberg E, Staten E, Watkins JC, McGraw D, McFadden C. 1976. A model of spinal cord injury. Surg Neurol 6:35–38.
4. Majkowski J. 1983. Drug effects on after discharge and seizure threshold in lissencephalic ferrets: an epilepsy model for drug evaluation. Epilepsia 24:678–685. https://doi.org/10.1111/j.1528-1157.1983.tb04630.x.
5. Raju SV, Kim H, Byzek SA, Tang LP, Trombley JE, Jackson P, Rasmussen L, Engellhardt JF. 2006. Cloned ferrets produced by somatic cell nuclear transfer. Dev Bio 293:439–448. https://doi.org/10.1016/j.ydbio.2006.02.016.
6. McCarron A, Donnelly M, Parsons D. 2018. Airway disease phenotypes related chronic bronchitis. JCI Insight 1:e87536.
7. Paquette SG, Banner D, Huang SS, Almansa R, Leon A, Xu L, Bartoszko J, Kelvin DJ, Kelvin AA. 2015. Impaired heterologous immunity in aged ferrets during sequential influenza A H1N1 infection. Virology 464–465:177–183. https://doi.org/10.1016/j.virol.2014.07.013.
8. Peng X, Alföldi J, Gori K, Eisfeld AJ, Tyler SR, Tisoncik-Go J, Brawand D, Law GL, Skunca N, Hatta M, Gasper DJ, Kelly SM, Chang J, Thomas MJ, Johnson J, Berlin AM, Lara M, Russell P, Swofford R, Turner-Maier J. 2018. Airway disease phenotypes related chronic bronchitis. JCI Insight 1:e87536.

TABLE 3 Current tissue repository

| Tissue            | Sample* | Sample forms                          | Sex       | Comment                      |
|-------------------|---------|---------------------------------------|-----------|------------------------------|
| Lung              | Brochioalveolar fluid                 | Single-cell suspension; homogenate; whole tissue; Trizol; paraffin-embedded tissue | M         | Influenza virus infected     |
|                   | Upper right, middle right, lower right, upper left, lower left | M and F Influenza virus infected and noninfected |
| Blood             | PBMC    | Fluid; isolated cells; RNAlater        | M and F   | Influenza virus infected     |
|                   | Plasma   |                                       | M         | and noninfected              |
|                   | Sera     |                                       | M         | Noninfected                  |
| Nasal fluid (wash)| NA       | Fluid                                 | M         | Influenza virus infected     |
| Spleen            | NA       | Whole tissue; single-cell suspension; homogenate; RNAlater | M and F   | and noninfected              |
| Trachea           | NA       | Whole tissue; single-cell suspension; homogenate; RNAlater | M and F   | and noninfected              |
| Mediastinal lymph node | NA | Whole tissue                          | M         | Influenza virus infected     |

*PBMC, peripheral blood mononuclear cells; NA, not applicable.
J. Young S, Hourlier T, Aken B, Searle S, Sun X, Yi Y, Suresh M, Tumpey TM, Siepel A, Wisely SM, Dessimoi C, Kawao K, Birren BW, Lindblad-Toh K, Di Palma F, Engelhardt JF, Palermo RE, Katze MG. 2014. The draft genome sequence of the ferret (Mustela putorius furo) facilitates study of human respiratory disease. Nat Biotechnol 32:1250–1255. https://doi.org/10.1038/nbt.3079.

16. Bruder CE, Yao S, Larson F, Camp JV, Tapp R, McBrayer A, Powers N, Granda WV, Jonsson CB. 2010. Transcriptome sequencing and development of an expression microarray platform for the domestic ferret. BMC Genomics 11:251. https://doi.org/10.1186/1471-2164-11-251.

17. Tisoncik-Jo G, Gasper DJ, Kyle JE, Elisfeld AJ, Selinger C, Hatta M, Morrison RL. 2016. Ferrets as a novel animal model for studying human respiratory infections in immunocompetent and immunocompromised hosts. J Virol 90:7991–8004. https://doi.org/10.1128/JVI.01002-15.

18. Kirchenbaum GA, Allen JD, Layman TS, Sautto GA, Ross TM. 2017. Vaccination of ferrets following influenza virus infection. PLoS Pathog 5:e1000642. https://doi.org/10.1371/journal.ppat.1000642.

19. Rutigliano JA, Doherty PC, Franks J, Morris MY, Reynolds C, Thomas PG. 2008. Screening monoclonal antibodies for cross-reactivity in the ferret model of influenza infection. J Immunol Methods 336:71–77. https://doi.org/10.1016/j.jim.2008.04.003.

20. Kirchenbaum GA, Ross TM. 2017. Generation of monoclonal antibodies against immunoglobulin proteins of the domestic ferret (Mustela putorius furo). J Immunol Res 2017:5874572. https://doi.org/10.1155/2017/5874572.

21. Kirchenbaum GA, Allen JD, Layman TS, Sautto GA, Ross TM. 2017. Infection of ferrets with influenza virus elicits a light chain-biased anti-IgM response in ferrets. Clin Vaccine Immunol 19:1798–2006. https://doi.org/10.1128/CVI.00247-10.

22. Erbelding EJ, Post D, Stemmy E, Roberts PC, Augustine AD, Ferguson S, Paules CI, Graham BS, Fauci AS. 2018. A universal influenza vaccine: the strategic plan for the National Institute of Allergy and Infectious Diseases. J Infect Dis. https://doi.org/10.1093/infdis/jiy103.

23. Li Y, Myers JL, Bostick DL, Sullivan CB, Madara J, Linderman SL, Liu Q, Carter DM, Wrammert J, Esposito S, Principi N, Plotkin JB, Ross TM, Ahmed R, Wilson PC, Hensley SE. 2013. Immune history shapes specific-antibody response against influenza A(H1N1) virus infection. J Exp Med 62:433–448. https://doi.org/10.1084/jem.148.3.674.

24. Cheng X, Zengel JR, Suguitan AL, Jr., Xu Q, Wang W, Lin J, Jin H. 2013. Evaluation of the humoral and cellular immune responses elicited by the live attenuated and inactivated influenza vaccines and their roles in heterologous protection in ferrets. J Infect Dis 208:594–602. https://doi.org/10.1093/infdis/jit207.