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Bongsup P. Cho, PhD


James F. Padbury, MD

Impact of NIH's Institutional Development Award (IDeA) Programs in Rhode Island

BONGSUP P. CHO, PhD
JAMES F. PADBURY, MD
GUEST EDITORS

The National Institute of General Medical Sciences (NIGMS) is one of the 27 Institutes and Centers that the National Institutes of Health (NIH) maintains. The NIGMS' Institutional Development Award (IDeA) program, which was formerly located in the National Center of Research Resources (NCRR) in the NIH, supports biomedical research, enhances investigators' competitiveness in securing research funding, student training, and enables clinical and translational research in states where NIH research funding levels have historically been low.[1]

The IDeA program has three primary mechanisms: (1) Centers of Biomedical Research Excellence (COBRE) that supports thematic, multidisciplinary research centers: (2) IDeA Networks of Biomedical Research Excellence (INBRE), a statewide biomedical research development network that partners research-intensive institutions with primarily undergraduate institutions (PUI); (3) Infrastructure for Clinical and Translational Research (IDeA-CTR) that supports clinical and translational research capacity. There is also an IDeA co-funding mechanism that provides a funding boost, and an STTR Regional Technology Transfer Accelerator Hub that supports infrastructure and builds an entrepreneurial network at IDeA institutions, one each in the four IDeA regions (Central, Northeastern, Southwestern, Western).

Rhode Island funding

Rhode Island (RI) has been very active in securing IDeA awards early on, receiving its first 5-year COBRE funding in 2000 for Brown University's COBRE Center for Genetics and Genomics. This was followed by a series of COBRE successes in RI's vibrant hospital research community: Rhode Island Hospital (COBRE Center for Cancer Research Development), Woman & Infants Hospital (COBRE for Perinatal Biology), and Roger Williams Hospital (COBRE Center for Stem Cell Biology). The trend has continued in recent years with a dozen newer COBREs focused on other innovative biomedical and translational themes (**Table 1**).

In 2001, the State was awarded its first exploratory Biomedical Research Infrastructure Network (BRIN) grant. It successfully transitioned to RI-INBRE in 2004 and has been renewed for four continuous 5-year grant cycles. The RI-INBRE award has enhanced and improved students' and faculty's ability to gain biomedical research opportunities in all eight network institutions (University of Rhode Island, Brown University, Bryant University, Providence College, Rhode Island College, Salve Regina University, Roger Williams University, the Community College of Rhode Island).[2] The latest in the IDeA category is Advance-CTR, which was awarded in 2016 to focus on the clinical and translational research program in collaboration with Brown University, the University of Rhode Island, and the area's healthcare systems. The translational mission of Advance-CTR complements RI-INBRE's biomedical focus, and together these comprehensive capacity-building programs have made outstanding contributions. Through funding, research resources trigger discoveries, improve health in the State and provides networking opportunities among researchers. For example, RI-INBRE's annual Summer Undergraduate Research Fellowship (SURF) conference is the largest of its kind in the State, featuring next-generation biomedical scientists. The Advance-CTR organizes the annual RI-IDeA Symposium that brings together investigators in the State to collaborate on IDeA-funded projects. Recently, RI-INBRE hosted a virtual seminar series, which highlighted RI-IDeA programs and recognized their impact in improving RI's biomedical research capacity. These IDeA programs have been truly transformative in building a vibrant community of biomedical and translational researchers. Faculty, students, and support staff at the various universities are now routinely networking and collaborating on their research projects. This is a seismic culture change in the academic research community, especially at the primarily undergraduate institutions.

Economic impact in RI

The economic impact of the IDeA support for the State is enormous. In 2020, RI received a total of 16 IDeA awards comprising RI-INBRE, IDeA-CTR, 12 COBRE, 2 IDeA Co-funding and INBRE-COBRE Collaborative Supplement, amounting to $32M (7.4% of the total IDeA funding) Additionally, RI is a leading site in the ECHO[3] (Environmental Influences on Child Health Outcomes) and ISPCTN[4] (IDeA State Pediatric Trials Network), a major NIH initiative to investigate the impact of environmental exposures on children's health and development, and to provide underserved and rural populations the opportunity to participate in high quality clinical trials.

In 2020, RI held the most COBRE grants among the IDeA states. It is worth noting that a significant portion of the

Table 1. Recent IDeA (Institutional Development Awards) Programs in Rhode Island

Name	Principal Investigator	Organization	Topic
COBRE Center for Addiction and Disease Risk Exacerbation (CADRE)	Peter M. Monti, PhD	Brown University	Addiction
COBRE Center for Central Nervous System Function (CCNSF)	Jerome N. Sanes, PhD	Brown University	Neuroscience
COBRE Center for Computational Biology of Human Disease (CCBHD)	David M. Rand, PhD	Brown University	Computational Biology
COBRE Center for Neuromodulation (CCN)	Benjamin D. Greenberg, MD, PhD	Butler Hospital	Neuroscience
COBRE Center for Antimicrobial Resistance and Therapeutic Discovery (CARTD)	Eleftherios Mylonakis, MD	Miriam Hospital	Infectious Disease
CardioPulmonary Vascular Biology COBRE (CPVB)	Sharon Irene Rounds, MD; Elizabeth Harrington, PhD	Ocean State Research Institute, Inc.	Pulmonary Cardiovascular
COBRE Stem Cells and Aging	Peter J. Quesenberry, MD	Rhode Island Hospital	Stem Cells and Aging
COBRE Center on Opioids and Overdose	Josiah D. Rich, MD, MPH	Rhode Island Hospital	Opioids, Overdose
COBRE for Reproductive Health	Surendra Sharma, MD, PhD	Women and Infants Hospital	Reproductive Health
Rhode Island IDeA Network of Biomedical Research Excellence (RI-INBRE)	Bongsup P. Cho, PhD	University of Rhode Island	Cancer, Environmental Health Sciences, and Neuroscience
Center for Clinical and Translational Science (Advance-CTR)	James F. Padbury, MD	Brown University	Clinical Translational
COBRE Center for Cancer Research Development (CCRD)	Bharat Ramratnam, MD	Rhode Island Hospital	Cancer
COBRE for Perinatal Biology	Sunil K. Shaw, PhD	Women and Infants Hospital	Perinatal Biology
COBRE Center for Immune-based Interventions Against Infectious Diseases	Alan L. Rothman, MD	University of Rhode Island	Infectious Disease
IDeA States Pediatric Clinical Trials Network (ISPCTN)	Phyllis Dennery, MD; Abbot Laptook, MD; Thomas Chun, MD, MPH	Rhode Island Hospital	Pediatric
COBRE for Skeletal Health and Repair	Qian Chen, PhD	Rhode Island Hospital	Musculoskeletal

IDeA funding is used to support research and related capacities, demonstrating its real impact, fueling discoveries, networking, and collaborations among institutions. For example, RI-INBRE (2001–2020) and Advance-CTR (2016–2020) have spent approximately 80% of their total funding directly on investigator-initiated research projects, student training, and associated core facilities. A similar impact is seen in COBRE grants. An additional example – during its entire grant period (2013–2020) the CardioPulmonary Vascular Biology Center of Biomedical Research Excellence (CPVB COBRE) spent approximately 85% of its funding on research pilot projects, junior investigator projects and core facilities. In addition, the IDeA programs over the years have supported research and administrative staff and undergraduate and graduate student training, directly contributing to the State's workforce and economy.

Biomedical IDeA programs

We requested each of the IDeA directors to provide a program description. In this Journal issue, we present contributions from 'basic science' biomedical research-oriented IDeA programs in RI. **CHRISTOPHER HEMME, PhD**, et al., describe the impact of RI-INBRE's capacity-building efforts in RI. **BENJAMIN D. GREENBERG, MD, PhD**, et al., discuss their new COBRE Center for Neuromodulation. **ALAN L. ROTHMAN, MD**, et al., highlight the significant impact of their Phase I COBRE in translational infectious diseases immunology. **NEILL Y. LI, MD**, and coworkers review the current understanding of how aging affects peripheral nerve regeneration following injury. **SURENDRA SHARMA, MD, PhD**, explains the arduous research journey from preeclampsia to Alzheimer's disease. **SHARON ROUNDS, MD**, et al., describe the capacity-building efforts in vascular biology in RI. **DAVID M. RAND, PhD**, and **ASHOK RAGAVENDRAN, PhD**, provide a program update on the COBRE Center for the Computational Biology of Human Disease at Brown University and affiliated hospitals.

In the subsequent April issue of the Journal, we will include the contributions from 'clinical' and 'translational' programs. Our goals are to make the clinical, biomedical, and scientific research community aware of the extraordinary resources that have been made available through these programs. We are blessed with this endowment of resources that have significantly enhanced biomedical enterprise in Rhode Island.

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Guest Editors

Bongsup Cho, PhD, Professor of Pharmacy, University of Rhode Island, Program Director of Rhode Island IDeA Network of Biomedical Research Excellence (RI-INBRE), Kingston, RI.

James Padbury, MD, Principal Investigator of Advance-CTR, William and Mary Oh - William and Elsa Zopfi Professor of Pediatrics for Perinatal Research, Brown University, Providence, RI.

Correspondence

Bongsup Cho, PhD

401-874-5024

bcho@uri.edu

RI-INBRE: A Statewide NIH Program Grant to Improve Institutional Biomedical Research Capacity in Rhode Island

CHRISTOPHER HEMME, PhD; LAURA BELLAVIA, MEd; SAMANTHA MEENACH, PhD; NIALL G. HOWLETT, PhD; BONGSUP P. CHO, PhD

ABSTRACT

The overarching goal of the Rhode Island-IDeA Network of Biomedical Research Excellence (RI-INBRE) is to improve institutional capacity for biomedical research excellence and expand student experiential training opportunities in the State of Rhode Island. RI-INBRE comprises five major core components: The Administrative Core, the Bioinformatics Core, the Centralized Research Core Facility, the Training Core, and the Developmental Research Project Program Core. Since its inception in 2001, RI-INBRE has made significant investments and marked advancements in the biomedical research infrastructure of Rhode Island. RI-INBRE funding has increased the scale and quality of faculty research and engaged undergraduate students, graduate students, and postdoctoral fellows in structured and mentored research training experiences. Over the last 19 years, RI-INBRE has supported 212 faculty researchers and over 533 projects and has provided research-training opportunities for nearly 2,000 students, resulting in 757 publications. Through its student-training program, RI-INBRE has contributed to regional workforce development by engaging students and encouraging them to pursue careers in biomedical fields. Many of these students have been admitted to graduate or medical schools and obtained biomedical industry jobs following graduation. RI-INBRE has been particularly influential in building the research infrastructure at primarily undergraduate institutions, which have seen significant improvements in research quality and output, student training, and research infrastructure.

KEYWORDS: biomedical research capacity, cancer, neuroscience, environmental health sciences

INTRODUCTION

The Institutional Development Award (IDeA) is a congressionally-mandated program administered by the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH). The IDeA Network of Biomedical Research Excellence (INBRE) program is designed to foster the development, coordination, and sharing of research resources and expertise, and to increase the number of competitive investigators in states where NIH research funding levels have historically been low.[1] While most institutions are eligible to participate in INBRE, an additional emphasis is on institutions such as primarily undergraduate institutions (PUIs) that typically lack the resources and research infrastructure of larger public and private institutions.[2] The goals of RI-INBRE include 1) financial support of early career scientists to help them achieve sustainable research programs, 2) provide resources to network institutions to increase research infrastructure through facilities upgrades and the hiring of research support staff, 3) to train undergraduate and graduate students and postdoctoral researchers with the goal of encouraging them to apply to graduate or medical school and/or find jobs in the biomedical sector, and 4) to enhance the local state and regional economies by providing a trained workforce in biomedical sciences.

The RI Biomedical Research Infrastructure Network (RI-BRIN) program began in 2001 and was replaced by RI-INBRE in 2004. The network includes the University of Rhode Island (URI), Brown University, associated local hospitals, and the Rhode Island (RI) PUIs: Bryant University, Providence College, Rhode Island College (RIC), Roger Williams University, Salve Regina University, and most recently the Community College of Rhode Island (CCRI). The funds allocated to these institutions support hiring of new biomedical faculty who can devote time to research, which has led to an increase in undergraduate student researchers and research productivity. In 2008 RI-INBRE began the Summer Undergraduate Research Fellowship (SURF) program to fund summer undergraduate research opportunities at the network institutions and local hospitals. This program has grown significantly in the years since and now constitutes an important training component of the RI-INBRE program.

STRUCTURE

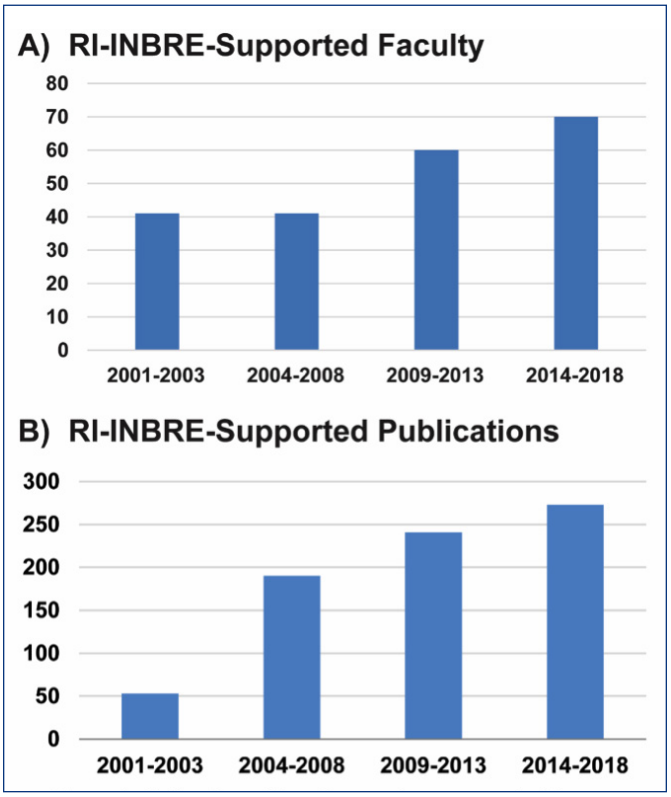
RI-INBRE is organized around several cores. The *Administrative Core* manages the administrative and financial aspects of the grant and is led by Program Director Bongsup Cho and Program Coordinator Niall G. Howlett and supported by Program Assistant Laura Arrighi and Program Business Manager Laura Bellavia. The major goals are to: manage the functions of the External Advisory Committee

(EAC), the Steering Committee (SC), and the Faculty Development Mentoring Committee; create a pipeline of trained individuals for careers in the cancer, neuroscience, and environmental health sciences thematic areas; and implement and oversee comprehensive internal survey and external assessment activities.

The *Developmental Research Project Program* (DRPP or Research Core) led by Program Coordinator Niall G. Howlett develops, solicits, and awards developmental research project funding to investigators at RI-INBRE network institutions (**Figure 1A**). The current scientific focus areas of RI-INBRE are cancer, neuroscience, and environmental health sciences. Research areas funded under these focus areas are broad and inclusive and include biochemistry and molecular biology, biomedical engineering, toxicology and natural products, psychology, neurodegenerative diseases, and environmental health sciences. A variety of support mechanisms are available to researchers depending on their career stage and specific needs. The primary funding mechanism of the Research Core is the Early Career Development (ECD) award. This is a 2–3-year award designed to support research program development of early career investigators at URI, Brown University, and the RI PUIs. ECD-supported investigators choose an experienced senior investigator to guide them in all aspects of research and professional career development over the course of the funding period. RI-INBRE also promotes innovative collaboration between well-established senior faculty at URI and Brown University and junior faculty at PUIs through the Collaborative Research (CR) award. This is a 2-year award that provides funding to both the junior and senior investigators. By partnering established investigators with junior PUI investigators, we aim to enhance the level of mentorship, thereby facilitating and accelerating research productivity and increasing the level of preparedness of the PUI investigators for submitting competitive independent or collaborative research proposals, e.g. NIH R15 or NSF RUI/ROA applications. The Research Core also supports pilot awards and summer undergraduate research fellowship (SURF) awards to faculty at the PUIs. Recent additions to the Research Core portfolio of funding opportunities include the Enhanced Virtual Education, RESearch, and Training (EVEREST) award to promote novel virtual/remote tools for education, research, and training, and Bioinformatics Pilot Project (BPP) vouchers to fund small-scale next-generation sequencing projects. Together, these mechanisms have supported over 500 faculty projects and led to a significant increase in research productivity as measured by scientific talks, poster presentations and publications (**Figure 1B**).

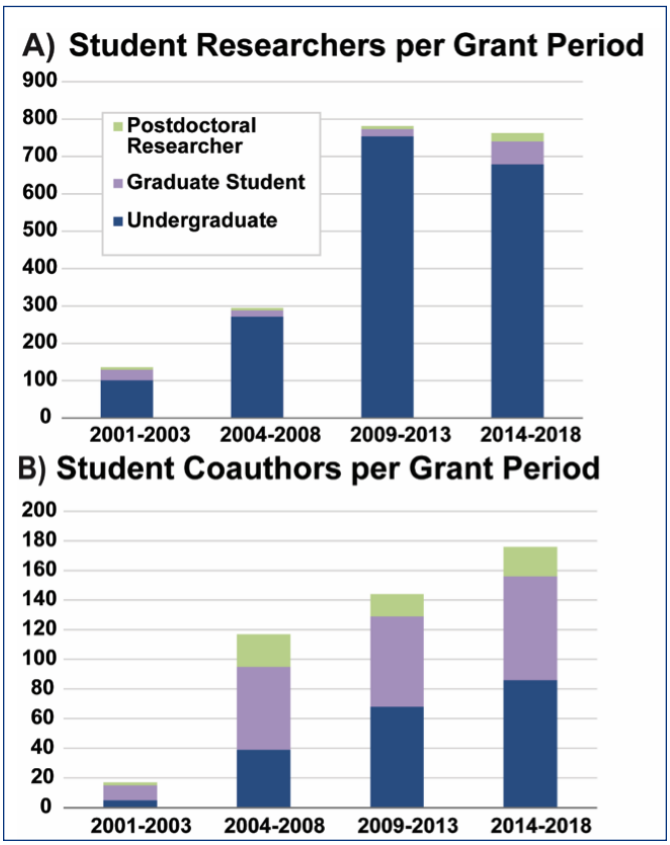
The goal of the *Training Core*, led by Dr. Samantha Meenach, is to establish a robust biomedical workforce pipeline in RI by providing research training opportunities for students, postdoctoral researchers, and occasionally faculty (**Figure 2**). The SURF program is the major component of this

Figure 1. A) Number of faculty members supported by RI-INBRE per grant period. **B**) Number of publications acknowledging RI-INBRE by grant period.



core and involves pairing undergraduate researchers with faculty mentors at URI, Brown University, and the PUIs to conduct research projects during the summer months. Students undergo training in research standards, lab safety, data handling, and other professional development topics. The program ends with a SURF conference where the students are given the opportunity to present their research to the community in a public poster session. The annual SURF conference is the largest of its kind in the state and draws approximately 400 attendees. Additional programs administered by the Training Core include the Bridges to Graduate School Program, which encourages RI-INBRE undergraduates to attend graduate school in RI, CCRI Summer Research Sabbatical Program, which places CCRI faculty in established labs at URI or Brown, giving them the opportunity to learn new skills to take back to their campus, and Teaching Postdoctoral Fellowships (TPF) Program. The latter is a partnership between RI-INBRE and the PUIs designed to advance the teaching and research mission of the PUIs, and to help train the next generation of teacher-scholars. Nearly 2000 students have been trained in the 19 years of the RI-INBRE program and student research productivity has increased in kind as measured by the growth in the number of student coauthors on manuscripts (**Figure 2B**).

Figure 2. A) Number and type of students supported by RI-INBRE per grant period. **B**) Number od students co-authors listed on RI-INBRE publications per grant period.



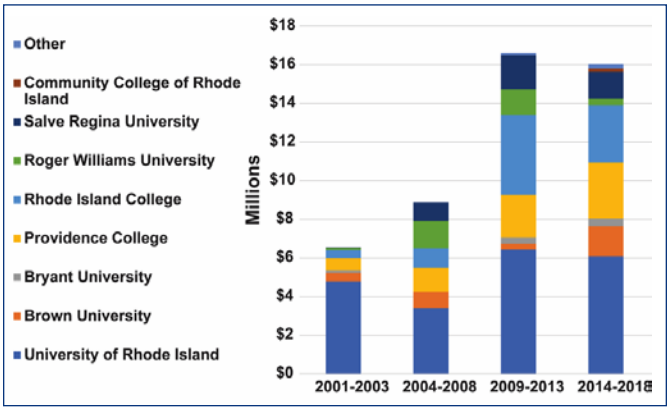
RI-INBRE also funds two core facilities to provide research services and support to the RI-INBRE and the state's biomedical community. The *Centralized Research Core Facility* (CRCF) based at the URI College of Pharmacy provides a central location for over $4 million of instrumentation, including microscopes, centrifuges, cell culturing, chromatography, and mass spectrometry. The CRCF is managed by Dr. Al Bach and Kim Andrews and provides training services free to RI-INBRE investigators and at subsidized rates to all other investigators. This facility is the only one of its kind in RI and is often cited by junior faculty as a significant resource in helping them establish and develop their laboratories. The *Bioinformatics Core* led by Dr. Christopher Hemme provides services and training in 1) bioinformatics and data science, 2) molecular modeling and other 3D science visualization tools such as 3D animation and projection, and 3) virtual and augmented reality applications.

DATA AND IMPACTS

From 2001 to 2024, RI-INBRE will have distributed $81 million dollars in research and training funding. As of 2020, this has supported 212 faculty researchers over 533 projects and the training of 1975 students. The research funded by the program has resulted in 757 publications with 176 undergraduate co-authors. RI-INBRE funding has been quite impactful in facilitating faculty research. For example, from 2001–2021, RI-INBRE spent 78% of its annual budget to support investigator-initiated research projects ($54M) (**Figure 3**). Through 2024, this number is expected to rise to $63M. RI-INBRE's impact is most dramatic at PUIs in terms of resources and research culture change. For example, RI-INBRE has provided Rhode Island College with over $10.0M in grants and equipment support since 2001. In 2001, faculty research and student training were not recognized as a requirement for achieving tenure and promotion at RIC and federal research funding, especially that provided by NIH, was rare. RIC faculty are currently securing ~$10M per year in external funding. In addition, hundreds of students are now participating in experiential research training during the academic year and summers. RI-INBRE funding has also contributed to facilities and infrastructure upgrades at PUI network institutions. Many of the faculty funded by RI-INBRE have graduated from the program by achieving research independence, often by being awarded NIH R01 and R15 and NSF RUI awards. Given the length of the RI-INBRE program, some former student researchers who are now independent scientists in RI have returned to the program as grant reviewers and research mentors for the next generation of student researchers.

Workforce development is a key goal of the RI-INBRE program. To assess the impact on the state and regional workforce, RI-INBRE staff have attempted to track students for at least five years to assess their career paths, whether they attended graduate or medical school, and whether they remained in the Northeast. Given the length of time the program has run and the large number of students, collecting and processing accurate data is a significant challenge. However, the data collected suggests a strong positive impact of the program on student career developments. Based on the sample of students we were able to track, >70% of

Figure 3. Monetary support of RI-INBRE network institutions by grant period.



undergraduate students who participated in the program are currently employed in the broader biomedical sector in the greater New England area (**Figure 3**). These jobs tend towards the pharmaceutical industry, but include biomedical engineering, chemistry, academic positions, entrepreneurs, and medical jobs (clinicians, nursing, pharmacy, etc.). Upon graduation, most students remained in Massachusetts, Rhode Island, and Connecticut. While most students ended their education with undergraduate (bachelor's or associate) degrees, data suggests ~25% of former RI-INBRE undergraduates went on to earn at least one graduate (masters or PhD) or medical degree. One indication of the improvement of research infrastructure at the PUIs is that many of these students were admitted to prestigious research programs around the country. All indications are that the program has been enormously successful at stimulating student interest in careers in the biomedical sciences and the retention of those students in Rhode Island and the Northeast.

PARTNERSHIPS

Collaboration between IDeA programs and networks is strongly encouraged by the NIH. Five states make up the Northeast IDeA region: Rhode Island, Maine, Vermont, New Hampshire, and Delaware. The Northeast INBRE programs frequently communicate to exchange ideas and determine best practices. Part of this effort includes the Northeast Bioinformatics Collaborative (NEBC), a collaboration between the five Northeast INBRE bioinformatics cores. This collaboration has included such efforts as the Northeast Cyberinfrastructure Consortium to enhance cyberinfrastructure for researchers in the Northeast and coordinated efforts at training through the Maine-New Hampshire Train-the-Trainer workshop, to train students and researchers in bioinformatics and data analysis skills. The NH-INBRE and DE-INBRE programs have been cooperating with RI-INBRE to provide sequencing services for the Bioinformatics Pilot Projects. To complement the mass spectrometry resources provided by RI-INBRE, the CRCF cooperates with the Oklahoma and Arkansas INBRE programs, both of which manage strong proteomics programs. In August 2021, RI-INBRE will host the biennial NorthEast Regional IDeA Conference (NERIC), bringing together all IDeA programs in the Northeast region to present research results, network, and build collaborations. Finally, many of the existing INBRE programs regularly cooperate on this issue of administrative data gathering and management for reporting and metrics tracking. Many of these programs utilize the PieStar software to manage reporting and eventually analysis of legacy data used to judge the effectiveness of the programs.

In addition to RI-INBRE, Rhode Island currently has 12 COBRE programs and an Advance-CTR program. Regular communication is maintained to identify synergistic activities between programs. The multiple core facilities periodically gather to discuss cooperative opportunities and needs of the RI IDeA programs. The Bioinformatics Core has individually cooperated with the Advance-CTR Brown Center for Biomedical Informatics (BCBI) and the COBRE Brown Center for Computational Biology of Human Disease (CBHD) on a variety of efforts. These efforts have included inviting speakers to URI from the BCBI, a metagenomics workshop with the CBHD, and the Rhode Island Microbiome Symposium 2020 with CBHD.[3] The symposium was an effort to bring together both environmental and clinical microbiome researchers from around RI and New England to discuss microbiome-related research. Over 100 researchers attended the conference and a follow-up conference will be organized once the COVID-19 pandemic has passed. This past summer, RI-INBRE organized a weekly virtual seminar series that involved the 12 IDeA program directors in RI. The series highlighted the state's capacity building and training programs and recognized the directors' valuable contributions in improving RI's biomedical research capacity. This virtual event was the first of its kind in RI and provided the state's biomedical community with great networking opportunities.

RI-INBRE has also established cooperative efforts with non-IDeA programs. Traditionally, the RI-INBRE SURF program collaborates with the NSF EPSCoR C-AIM to hold a single SURF conference at the end of each summer where all SURF participants are given the opportunity to present their research to the community. This year the RI-INBRE SURF program welcomed Maximizing Access to Research Careers (MARC) Undergraduate Student Training in Academic Research (U-STAR) program trainees to participate in professional development and research training activities and to present their data at the annual SURF symposium. The MARC U-STAR program is a research and professional development program supported by NIGMS for undergraduate students from underrepresented backgrounds.[4]

To promote entrepreneurship and intellectual property development by RI-INBRE researchers, RI-INBRE collaborates with the NIH-funded Northeast DRIVEN Acceleration Hub and RI Bio life sciences industry group to share best practices and provide workshops, webinars, forums, and resources to the RI's innovative and entrepreneurial community.

FUTURE EFFORTS

The RI-INBRE program has undergone significant changes since its inception 20 years ago and has had a truly transformative effect on RI's biomedical research community. The program has inspired a culture change among researchers, especially at the PUIs, launching and supporting the independent careers of multiple faculty and promoting new collaborations across all the RI colleges and universities. The two goals of student training and junior scientist support will

remain priorities in the future. We will continue to adapt and innovate moving forward. The RI-INBRE program is also committed to promoting a diverse, equitable, and inclusive biomedical research culture across the state of Rhode Island. The program actively supports and promotes the hiring of both faculty and postdoctoral research fellows from diverse/unrepresented backgrounds at the RI PUIs and will continue to support and lead statewide efforts to diversify the biomedical and scientific workforce. A centralized data reporting and tracking system will simplify annual reporting and metrics tracking and will provide an accurate assessment of trends within the RI-INBRE program and between INBRE and other IDeA programs. New initiatives such as the EVEREST and BPP funding mechanisms will expand the scope of existing research funding mechanisms allowing the maximum number of faculty to participate and stimulating new ways to train the next generation of biomedical researchers. The program will continue to recruit quality students and engage them in research activities while encouraging them to pursue biomedical careers following graduation. Ultimately, these activities will continue to greatly enhance the biomedical infrastructure of Rhode Island, meeting the primary goal of the IDeA INBRE mechanism.

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Authors

Christopher L. Hemme, PhD, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI.

Laura Bellavia, MEd, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI.

Bongsup P. Cho, PhD, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI.

Samantha Meenach, PhD, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy and Department of Chemical Engineering, College of Engineering, University of Rhode Island, Kingston, RI.

Niall G. Howlett, PhD, Department of Cell & Molecular Biology, College of Environmental and Life Sciences, University of Rhode Island, Kingston, RI.

Correspondence

Bongsup P. Cho, PhD
bcho@uri.edu

The COBRE Center for Neuromodulation (CCN) at Butler Hospital: Clinical-Translational Research in Human Brain Stimulation

BENJAMIN D. GREENBERG, MD, PhD; NOAH S. PHILIP, MD; KRISTEN FORTIN-ASHBURNE, MBA; LINDA L. CARPENTER, MD

ABSTRACT

The COBRE Center for Neuromodulation (CCN) at Butler Hospital supports clinical research in neuromodulation and investigators' career development in this field. The work couples brain stimulation methods with readouts of brain activity (e.g., using various neuroimaging, behavioral, and physiological assessment methods) in clinical or clinically relevant populations. Its guiding principle is that for noninvasive brain stimulation to gain efficacy and implementation, it is essential to better characterize clinically relevant target circuits and mechanisms of action. The CCN includes a Design and Analysis Core (DAC) to support rigorous and innovative experimental design and data analytic strategies and a Neuromodulation and Neuroimaging Core (NNC) to facilitate the acquisition and processing of high-quality data using noninvasive neurostimulation and neuroimaging methods. This article will describe the CCN's research focus and how it enhances research capacity in neuromodulation in our state. It will introduce our current investigator Project Leaders, their projects, and our pilot project program. It will also detail the CCN's links to Centers and research cores in Rhode Island researching allied areas of clinical neuroscience, neurology, psychiatry, and psychology, current collaborative efforts across those centers, and opportunities to collaborate in research and training.

KEYWORDS: neuromodulation, TMS, tTDS, neuroimaging, neuronavigation

INTRODUCTION

To quote this issue's Introduction, "the impact of behavioral and mental health problems on the overall health of individuals and populations has become increasingly recognized" (Padbury and Cho). Neuromodulation is typically used in combination with pharmacotherapies and psychotherapies to address the burdens mental illness (and other brain disorders) impose on individuals, families, and population health. Here, we use CCN members' work to illustrate important aspects of the therapeutic and research contexts of neuromodulation. And we highlight the new infrastructure and resources that the CCN has made available to enhance work in this promising area, and to help train and support the new generation of neuromodulation researchers in Rhode Island.

"Neuromodulation" might be any method modifying the nervous system. So, medications, devices, psychotherapies, mind-body approaches, and more might qualify. The term, however, has a narrower definition: device-based methods affecting the CNS. Typically, devices emit energy (e.g. electrical, magnetic, ultrasonic) to modify brain activity directly or via peripheral nervous system components. This definition of neuromodulation dovetails with the use of brain networks as a fundamental unit of analysis in understanding brain-behavior relationships in health and disease. A network can be thought of as a collection of nodes that are structurally and functionally related. This, in turn, lends itself to translational research involving an exchange between cognitive and affective neuroscience and clinical disciplines. Models of illness pathophysiology envision dysfunction at multiple levels: within regions, within networks, and in the smooth flow of information across networks. For an example of how brain functional architecture might relate to illness symptoms (in this case of post-traumatic stress disorder, PTSD). see Barredo et al.[1] Regarding how neuromodulation might be understood to act at the level of brain networks in PTSD and major depression, see Philip et al. [2]

Another translational aspect of neuromodulation is using clinically relevant affective or cognitive processes as the basis for research (complementing the focus on anatomical networks). Examples include studies by CCN Project Leaders, e.g., by Nicole McLaughlin on response inhibition in obsessive-compulsive disorder[3], or Mascha van't Wout-Frank and colleagues on affective elements in decision-making. [4] The abilities to flexibly inhibit responses, or to incorporate the experience into decision-making, are essential to adaptive behavior in health or illness. Another example is using brain stimulation to augment extinction of conditioned fear, thought to underlie therapeutic change in PTSD.[5] These studies represent essential steps guiding development of brain stimulation methods for eventual clinical use, and will generate results that advance fundamental knowledge in cognitive and affective neuroscience.

Neuromodulation is seeing the application of increasingly powerful tools, both in the array of devices used and their combination with neuroimaging, electrophysiology, and computational modeling to understand impacts of

stimulation on brain and behavior. Mechanistic insights, in turn, guide research refinement, with each next step addressing goals and hypotheses relevant to health and therapeutics. These include identifying specific brain regions, circuits, or oscillatory signals to be engaged and modulated; establishing efficacy and safety of a neuromodulatory treatment at a group level; selecting specific stimulation methods for individuals most likely to benefit; tailoring neuromodulation to optimize effects in each participant or patient; and developing technologies and implementation steps required for widespread dissemination of a new treatment to meet the large and manifest clinical need. This research can exemplify multiple stages along the translational research continuum, and its success requires an ongoing interplay between clinicians, clinical researchers, basic and cognitive neuroscientists, and biomedical engineers.

Devices used can be invasive (implanted) or noninvasive. An example of the former is deep brain stimulation, currently used clinically in Parkinson's disease, essential tremor, dystonia, and obsessive-compulsive disorder (OCD; see Greenberg, et al.[6] for the latter). The CCN's focus, in contrast, is noninvasive methods. The best-known noninvasive therapy is transcranial magnetic stimulation (TMS, also 'repetitive TMS'), a standard-of-care treatment for severe major depressive disorder (MDD). The other non-invasive method we will describe here, which thus far has not gained regulatory approval for clinical treatment, is transcranial direct current stimulation (tDCS).

TRANSCRANIAL MAGNETIC STIMULATION

Here, a pulsed magnetic field delivered through the scalp and skull stimulates relatively superficial brain. The magnetic flux generates electrical activity in the cerebral cortex, producing both local and, via networks, distant effects. TMS is "supra-threshold," meaning that enough energy is delivered to induce action potentials in target neurons. TMS research in MDD began in the early 1990s, with subsequent development through extensive clinical trials. Results converged on the effectiveness of TMS. In practice, patients come to the clinic for repeated TMS therapy sessions, delivered in each session as brief "trains" of pulses with rest between trains. Typically these are once per weekday for up to six weeks, often followed by several weeks during which TMS is tapered. See Carpenter et al.[7] for an example of research on clinical effectiveness and also a recent update on TMS in depression.[8]

During each session, a patient receives many TMS pulses over a defined region, usually left or right dorsolateral prefrontal cortex. Researchers continue to develop new devices, one of many examples being a device using a multiple electromagnetic coil array[9] instead of the single-coil design of all currently approved devices. Novel approaches for selecting stimulation parameters and new methods for identifying the best brain targets are under investigation, many informed by electroencephalography and neuroimaging findings generated by senior CCN researchers. TMS treatment protocols have shown preliminary clinical efficacy for numerous neuropsychiatric and behavioral disorders; randomized clinical trials recently led to the US FDA approvals for TMS to treat OCD and for smoking cessation. TMS is increasingly available across healthcare systems in the United States and elsewhere.

In Rhode Island, there are active neuromodulation clinical programs (or TMS clinics) at Butler Hospital, the Providence VA Medical Center, and Rhode Island Hospital, with several others in private-practice settings. Despite increasing public awareness that TMS therapy is an option for patients who do not achieve adequate benefit with more traditional depression treatments, it has remained underutilized since its 2008 FDA approval. The substantial time commitment required may be one reason for slow uptake. Recent research has led to the approval of a novel stimulation pattern, intermittent "theta-burst" TMS, which can be delivered in the clinic much more rapidly than "traditional" TMS (2-3 minutes rather than 20-30 minutes per session). Research applying the theta burst TMS method to other conditions, like PTSD, has generated substantial interest.[10]

TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS)

tDCS involves low-energy electrical current, following the principle that applying current to the skin generates an electrical field. Although there are commercially available devices for transcranial electrical stimulation (largely marketed directly to consumers), here, we restrict ourselves to tDCS, which delivers a constant ("direct") current waveform. See Philip et al.[11] for a comprehensive review of tDCS and related methods. Devices deliver a predefined intensity of electric current of several milliamperes, using a variety of electrode arrays for current "focusing." For example, a single anode typically using multiple smaller cathodes around a single anode (or vice versa), for more focal tDCS. While the skin and skull are transparent to the magnetic pulses delivered by TMS, there is resistance to energy flow between electrodes during tDCS. The electrical fields used in tDCS are considered subthreshold, i.e., stimulation at the intensities used does not independently cause neuronal depolarization. Rather, it is thought to bias neurons' activity to influence the ongoing flow of information in a pathway. The factors involved are complex, but the basic point is that even a small tDCS-induced change in membrane voltage may impact activity. An example is a pilot study by CCN investigators who evaluated tDCS concurrently with psychotherapy in depression. Most tDCS devices are relatively inexpensive, have a good safety profile, and can be deployed either in a clinical research laboratory or in a nonresearch

environment; recently tDCS devices have been designed for self-administration at home, with remote "supervision" functions to support investigation of their effects in randomized controlled trials comparing active or sham stimulation.

THE COBRE CENTER FOR NEUROMODULATION (CCN) AT BUTLER HOSPITAL

The CCN (https://www.butler.org/services/cobre/) was established in 2019 as part of the NIH-NIGMS-funded IDeA (Institutional Development Award) program. CCN has a primary focus on device-based neuromodulation, and particularly that using noninvasive methods. A guiding principle of our Center is that for neuromodulation methods to gain acceptance and implementation by the clinical community, it is necessary to understand their mechanisms of action (requiring clinical-translational research) and their therapeutic efficacy (the domain of clinical trials). That, in turn, opens an interplay between our understanding of mechanisms underlying impairments in mental health or neuropsychiatric conditions more broadly. These can either be understood as traditional diagnostic categories (a categorical diagnosis requires that an individual "meets criteria" for an illness; the person either has it or not) or be based on a dimensional view of maladaptive behavior. In the latter view, clinically significant problems emerge when actions that occur along a continuum in the general population are expressed with problematic severity or frequency.

Our Center's components is overseen by the CCN Administrative Core, aided by three advisory committees. One of these committees' overall work is based at the home institution, Butler Hospital. Its members are leaders in various hospital departments required to facilitate and support core operations of the CCN; the second committee also includes representatives of local research institutions, and finally, an External Advisory Committee composed of leading national experts in neuromodulation research. The CCN Administrative Core works closely with our program partners in the state.

CCN is a "Phase 1" COBRE, built around multiyear projects in human neuromodulation, each led by a Project Leader developing a research career. Currently, there are four Project Leaders, each advised by mentors providing scientific, technical, and career guidance. The projects and leaders are: 1) Brain Circuitry of inhibitory control in young adults: Modulation with tDCS, Nicole C. McLaughlin, PhD; 2) Effects of tDCS timing on safety memory in PTSD, Mascha van't Wout-Frank, PhD; 3) Modulating prefrontal circuits underlying behavioral flexibility in OCD: a TMS study, Sarah Garnaat, PhD; and 4) Determining whether TMS changes the brain through brain synaptic plasticity, Joshua Brown, MD, PhD.

The projects are supported and facilitated by two CCN research cores. The cores are also designed to serve a separate Pilot Project Program, which allows faculty researchers at Rhode Island institutions to collect preliminary data in clinically relevant neuromodulation studies to support external grant proposals. The Design and Analysis Core (DAC; Director Richard Jones, PhD; Co-Director Jennifer Barredo, PhD), as its name implies, supports the experimental design, data processing methods, and general statistical aspects appropriate to human subject's research. Given that understanding possible mechanisms underlying effects of neurostimulation on the brain and behavior is central to the mission of CCN, this core also specifically addresses needs particular to neuroimaging and neurophysiology research (e.g., fMRI, brain connectivity or evoked responses measured with EEG or electromyography). A new high-capacity CCN computing center at Butler Hospital houses dedicated workstations for MRI and for EEG analyses with internet access, software, external storage devices, and direct connection to a dedicated data storage server.

The aspects of "hands-on" brain stimulation training and data acquisition are the Neuromodulation and Neuroimaging Core (NNC, Director, Linda Carpenter, MD; Co-Director, Noah Philip, MD). The NNC hosts an array of TMS equipment, including several different devices and "neuronavigation" platforms that permit stimulation to particular brain regions with an individual's MRI. There are TMS coils for both active and sham stimulation to permit controlled studies. The TMS coils themselves have different designs producing different magnetic field shapes for stimulating brain regions that differ in location and extent, and the TMS stimulators have various features as needed for the application of theta-burst stimulation or electrophysiological studies of cortical excitability; the latter is used in combination with pharmacological probes to investigate plasticity mechanisms of TMS action (see Brown et al.[12,13]) Similarly, there are multiple tDCS devices available in the core, allowing investigators to select different electric field geometries as needed for their research purposes. There are also computational modeling platforms available via the NNC, which use neuroimaging and/or brain models to design the optimal montage for the desired stimulation effect with either TMS or tDCS; such models ensure the resulting electrical current will engage particular brain targets with a degree of selectivity. The NNC also supports EEG recording with several research-grade electrophysiology platforms, with additional modules and accessories to permit collection of other psychophysiological data (e.g., cardiac data, galvanic skin response, and other physiological responses). Both the NNC and DAC work closely with the Brown MRI Research Facility to support the collection of high-quality neuroimaging data in concert with neuromodulation procedures.

It is to our great advantage that senior researchers at CCN also lead active neuromodulation clinical programs in Rhode Island, the TMS Clinic at Butler Hospital, directed by one of the authors (Carpenter), and the Neuromodulation Clinic at the Providence VA Medical Center, led by another

(Philip). The NNC is, in fact, adjacent to the space used by the Butler TMS Clinic. This has allowed it to draw upon and utilize additional clinically trained staff support and other resources from Butler's TMS clinical service. Researchers in the core facility at Butler thereby have immediate access to the infrastructure of the TMS Clinic and other clinical service resources including a pool of physicians, technicians, and a nurse trained in TMS and other neuromodulation techniques, access to medical equipment, emergency response teams, and physician supervision for management of potential adverse events as well to address any clinical issues that may arise in the patient populations under study. This arrangement allows our Center and the NNC specifically to be an essential resource for the safety and success of projects involving clinical samples, particularly in cases where the principal investigator is not medically trained and/or does not have access to neurostimulation-trained physicians on his or her research team. The two research cores collectively host training in brain stimulation and neuroimaging methods, including the safe and experimentally rigorous use of brain stimulation devices, MRI, EEG, data acquisition, data management, quality control and analytic methods.

CCN's cores have worked hard during the COVID-19 pandemic to implement procedures ensuring safe research in this evolving environment, a complex and ongoing task involving multiple institutions and facilities, and IRBs. Our personnel's dedication has enabled research protocols to be adapted flexibly as needed, and to be restarted as soon as possible after a pandemic-imposed pause. We share tremendous optimism regarding our Center's capacity to enhance research in this exciting and rapidly developing field, and welcome interest and engagement by the research community.

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Authors

Benjamin D. Greenberg, MD, PhD, Butler Hospital, Providence RI; Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University, RR&D Center for Neurorestoration and Neurotechnology, Providence VA Medical Center.

Noah S. Philip, MD, Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University, RR&D Center for Neurorestoration and Neurotechnology, Providence VA Medical Center.

Kristen Fortin-Ashburne, MBA, Butler Hospital, Providence, RI.

Linda L. Carpenter, MD, Butler Hospital, Providence RI; Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University.

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Correspondence

Benjamin D. Greenberg, MD PhD
benjamin_greenberg@brown.edu

Immune-Based Interventions Against Infectious Disease – Impact of a Phase I Center for Biomedical Research Excellence in Translational Infectious Diseases Immunology

ALAN L. ROTHMAN, MD; JENNIFER FRIEDMAN, MD; JONATHAN D. KURTIS, MD, PhD

ABSTRACT

In 2011, faculty from the University of Rhode Island (URI)'s Institute for Immunology and Informatics and Lifespan's Center for International Health Research collaborated to develop a successful application for a Phase I Center of Biomedical Research Excellence around the scientific theme of translational infectious diseases immunology. From 2013 to 2020, this COBRE supported significant discoveries in research on dengue, HIV, and malaria, among other diseases, and facilitated the career development of several independent Rhode Island (RI)-based early-stage investigators. Our experience illustrates both the potential and challenges for investigators with shared scientific interests to leverage the NIH COBRE program to enhance cross-institutional interactions.

KEYWORDS: immunology, infectious diseases, global health, translational research, host-pathogen interactions

INTRODUCTION

Research on infectious diseases is essential both in the U.S. and globally. Well-known infectious diseases continue to cause significant morbidity and mortality, and new and re-emerging infectious disease threats are being increasingly recognized, most recently exemplified by the COVID-19 pandemic.[1,2] The diversity of human pathogens requires a broad research strategy, including efforts to utilize or enhance innate and adaptive immune mechanisms for preventive strategies and as broad-spectrum therapeutics. There is also increasing attention to the need to modernize the structure and organization of scientific investigation.

The University of Rhode Island (URI), the state's primary public research university, lacks a medical school, and its infectious diseases research is divided among several colleges. In 2009, URI founded the Institute for Immunology and Informatics (iCubed) in the Biotechnology Center on the Providence campus, with a research mission in the immunology of human infectious diseases. Brown University has the state's only medical school. Its infectious disease and immunology research is spread across multiple departments and programs within the medical school and its affiliated teaching hospitals. In 2005, Rhode Island Hospital founded the Center for International Health Research (CIHR), an interdepartmental center with a research mission on immunity to malaria and schistosomiasis. These two centers were thus both actively conducting NIH-funded translational infectious diseases immunology research in downtown Providence. At the same time, both groups faced challenges associated with a small size and distinct mission, particularly limited resources to expand strategically into new research opportunities, provide career advancement for young scientists, and fund the purchase of advanced equipment.

The common scientific interests and philosophy and the proximity of iCubed and CIHR led us to institute a combined Journal Club/research-in-progress meeting and initiate discussions in 2011 regarding potential collaborative opportunities. The NIH Centers for Biomedical Research Excellence (COBRE) program provided an attractive solution. In contemplating a collaborative COBRE proposal, we defined two key priorities and objectives: a) achieving a "critical mass" of at least 8–10 full-time funded investigators with related scientific interests to make the groups competitive for institutional training grants and shared instrumentation grants; and b) creating a sustainable mechanism to promote career advancement of junior investigators and launch new research initiatives.

CONCEPT FOR THE PHASE I COBRE

In our COBRE Phase I application, "Immune-Based Interventions Against Infectious Diseases," we outlined a vision to build an entrepreneurial, multidisciplinary, and trans-institutional research team to address infectious diseases of global importance. The COBRE proposed to catalyze the

Figure 1. Logo for our COBRE focused on infectious diseases immunology. The logo highlights COBRE-supported research with images of dengue virus, a mosquito vector, *Plasmodium falciparum*-infected red blood cell, and human immunodeficiency virus (clockwise from upper left). The world map in the center highlights the focus on global health, and the subtitle highlights the multi-institutional collaboration.

partnership between the vital independent research programs in iCubed and CIHR around the unifying scientific theme of translational research in pathogen-host interactions (**Figure 1**).

This proposed COBRE's significance and innovation lay in its intent to pursue transformational advances in infectious disease immunology research, bypassing traditional paradigms of departmental growth to address high-priority research objectives. Distinctions from existing research programs in RI included: 1) applied research, 2) a global health focus, 3) interdisciplinary research including both MDs and PhDs, and 4) an emphasis on collaboration, shared governance of common resources, and recognition of faculty teamwork.

URI/iCubed served as the primary grantee for the COBRE, with a substantial component at RI Hospital/CIHR (**Figure 2**). Leadership was provided by a senior Program Director, a Steering Committee of established URI and Lifespan faculty, and an External Advisory Committee of 5 senior NIH-funded MD and PhD investigators. Support was solicited from the broader RI community of infectious disease and immunology researchers; affiliated faculty members were asked to participate in conferences and seminars, and in turn, would have priority access to COBRE core facilities.

Our Phase I COBRE proposed four Specific Aims:

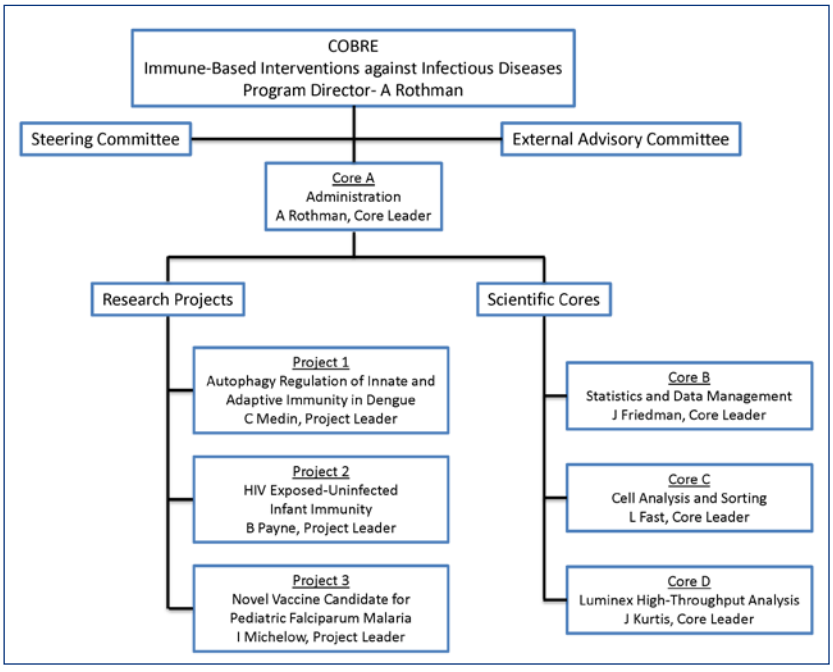
Aim 1 was to provide leadership to the RI research community in translational infectious diseases immunology. Visibility and identity for the COBRE would be established through support for conferences and pilot projects. These had already been accomplished on a smaller scale at iCubed, including through R13 and U19 grants. Support for conferences would include the established Vaccine Renaissance Conference, Journal Club, seminars, and an annual Research Retreat. The Pilot Projects program would support promising early-stage investigators and new interdisciplinary collaborations.

Aim 2 was to build infrastructure for immunology research. COBRE-supported scientific cores (**Figure 2**) were designed to provide CORBE investigators with the tools to generate and manage complex immunologic datasets. Our efforts focused on platforms with high-throughput capacity and multiparameter read-outs. The Cell Analysis and Sorting Core would provide immunoassay, flow cytometry, and cell sorting services focused on automation, throughput, and biosafety. The Core would leverage existing equipment (e.g., LSR II analyzer and AutoMACS) and would fund the purchase of a new Miltenyi MACSQuant Analyzer. The Core would focus on training and advising research staff and facilitating their use of the equipment. The Luminex High-Throughput Analysis Core would provide assays on an existing Luminex platform in a high-throughput mode. Under COBRE support, this equipment would be available for use by a more extensive cadre of researchers. Additional sample handling equipment would be acquired to increase assay capability. The Statistics and Data Management Core would support COBRE investigators in managing and analyzing these and other research data. The Core would assist COBRE investigators with managing complex datasets and performing sophisticated data analyses incorporating clinical predictors and outcomes collected in the field. The Core would also serve an educational role by providing both project-specific, tailored advice and broader education in epidemiology, data management, and biostatistics to facilitate communicating with collaborators, analyzing complex data, and writing grants.

Aim 3 was to recruit and mentor promising junior investigators. The strategic plan for the growth of the COBRE involved the direct investment in three junior investigators and new investigators' recruitment. Mentoring of these individuals was critical to this plan. We proposed a detailed mentoring plan, including the designation of a primary mentor and co-mentor for each of the junior investigators. Milestones and timelines were defined, with plans for regular meetings and evaluations, to be reviewed by the Steering Committee and External Advisory Committee.

Aim 4 was to support translational infectious diseases immunology research by junior investigators. Our COBRE proposal identified three junior investigator-led projects that would build on established iCubed and CIHR research

Figure 2. COBRE organizational chart at start of Phase I funding period. A fourth Research Project was added in Year 2.



programs but introduce novel study populations or models and new research techniques (**Figure 2**). "Autophagy Regulation of Innate and Adaptive Immunity in Dengue," led by Dr. Carey Medin, focused on host cell responses to dengue virus (DENV). This project proposed to analyze the induction of autophagy by DENV and its effects on innate immune signaling and activation of T lymphocytes by DENV-infected antigen-presenting cells. The significance of the project lay in the expanding global impact of dengue and the potential relevance of autophagy to dengue disease pathogenesis. Translational aspects of this project included the analysis of primary human cells and clinical isolates of DENV. "HIV Exposed-Uninfected Infant Immunity," led by Dr. Barbara Payne, focused on the impact of in utero exposure to HIV on development of the immune system. This project proposed to compare the repertoire of T lymphocytes in HIV-uninfected infants born to HIV-infected or uninfected mothers and define the maternal characteristics that affect the development of the infant immune system and the effects of a narrowed T cell repertoire on subsequent immune responses to infant vaccinations. The significance of the project lay in the increased risk for morbidity and mortality in infants of HIV-infected mothers despite preventing maternal-fetal transmission of HIV. Translational aspects of this project included the analysis of clinical specimens and clinically relevant outcomes. "Novel Vaccine Candidate for Pediatric Falciparum Malaria," led by Dr. Ian Michelow, focused on defining the mechanisms of protection by antibodies to a novel malaria antigen. This project proposed to analyze the expression of this antigen and its association with protection. The significance of the project lay in the need to identify protective malaria antigens for vaccine development. Translational aspects of this project included the focus on vaccine development and the use of clinical specimens.

EXECUTION AND OUTCOMES OF PHASE I COBRE ACTIVITIES

Through Phase I COBRE funding, iCubed and CIHR generated new and productive research collaborations, recruited additional investigators, and expanded the reach of these collaborations within RI.

Under Aim #1, iCubed and CIHR solidified the weekly joint research-in-progress/journal club meeting, which expanded to include several Pilot Project investigators and new hires. We also organized three local retreats/workshops bringing together RI-based academic and industry investigators in immunology, infectious diseases, and bioinformatics/big data, which served to catalyze several collaborative research and teaching initiatives. With an institutional contribution to the program from Lifespan, the COBRE Pilot Projects program supported a total of 14 projects over the phase I period (**Figure 3**). In addition to supporting junior investigators within iCubed and CIHR, pilot project funding

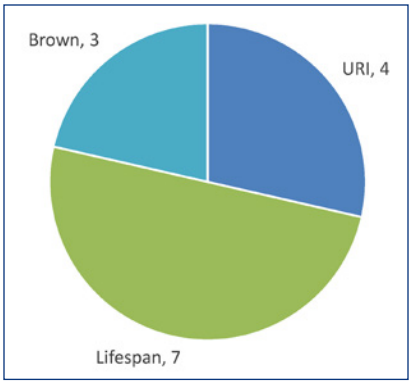


Figure 3. Distribution of funding from the COBRE Pilot Project program, by home institution (N = 14). Direct COBRE support for 10 pilot projects was supplemented by funding for 4 projects from Lifespan.

supported investigators in other units within URI (Pharmacy, Cell and Molecular Biology), Lifespan (Gastroenterology, Pediatrics, Oncology, Infectious Diseases), and Brown University (Molecular Microbiology and Immunology). Overall, these activities succeeded in raising the profile of infectious diseases immunology research within RI and encouraged new and interdisciplinary research collaborations. The COBRE triggered stronger interactions between URI and Brown University and its affiliated hospital systems that are an ongoing positive outcome of the COBRE.

Under Aim #2, COBRE funding expanded the laboratory infrastructure and made these resources available to the local scientific community through CoresRI.org and shared scheduling calendars. The Data Management and Statistics Core served as a model for other programs. Its personnel were recruited to participate in several other RI COBREs, and the Brown University Environmental influences on Child Health Outcomes (ECHO) program. Equipment added to the Cell Analysis and Sorting Core and the High-Throughput Luminex Immunoassay Core continues to support research at URI, Lifespan, and Brown University.

Under Aim #3, we recruited additional faculty members to iCubed and CIHR and paired younger investigators with senior mentors. During the phase I period, we recruited five new faculty investigators, two at iCubed and three at CIHR. One of these investigators was recruited using COBRE Research Project support, and three of the other investigators received COBRE pilot project funding. All of the Research Project Leaders and nearly all Pilot Project investigators submitted applications for external grant funding. Several Pilot Project investigators successfully transitioned to independent status, and others received smaller NIH or foundation grants as PI. Two Research Project leaders were promoted within their respective institutions.

Under Aim #4, we supported a total of four full Research Projects and 14 Pilot Projects during the Phase I period. All of the Research Project leaders had success at obtaining some external funding for their research; two achieved "independent" status as overall or local PI on an NIH R01 grant. Overall, 13 published manuscripts from Research Project leaders cited COBRE support. The Pilot Projects program

contributed to 4 R01s, 5 R21s, at least 15 smaller (e.g., foundation) grants, and 17 additional published manuscripts.

Major scientific outcomes from COBRE Research Projects included the following:

- COBRE research described a novel system for live-cell imaging of DENV-infected cells and demonstrated that DENV infection resulted in the suppression of mitochondrial fission through a reduction in intracellular levels of dynamin-related protein 1.[3,4] These findings identified a novel effect of DENV on cell function and provided insights into dengue pathogenesis.
- COBRE research demonstrated that maternal HIV infection was associated with elevated serum cytokine levels, elevated levels of cytokines in the cord blood of their HIV-exposed, uninfected (HEU) infants, and reduced T cell receptor beta chain gene diversity in a subset of these infants.[5,6] These findings demonstrated that maternal HIV infection affected in utero development of the immune system in HEU infants, potentially contributing to the higher risk of illness in these infants.
- COBRE research characterized a novel malaria parasite antigen expressed both on the parasite surface and on the surface of infected RBCs selectively recognized by cohort subjects at lower risk of illness. These findings identified a novel candidate for vaccines to prevent malarial disease.
- COBRE research identified malaria parasite antigens selectively recognized by individuals with low/undetectable gametocytaemia.[7] These findings point to a vaccination strategy to block parasite transmission.

CHALLENGES

Characteristics that made our Phase I COBRE unusual unfortunately also created challenges. Our COBRE aimed to catalyze a collaborative effort between groups at two different institutions that was at a very early stage. None of the senior or junior COBRE investigators held tenure-track faculty positions. The research projects targeted for support were at a very early phase, as COBRE support provided the resources to launch these independent research projects. While we envisioned the COBRE program as an excellent fit for our objectives, our model did not include more established (yet still early-stage) investigators and projects who could provide the "quick success" that some COBREs rely on and NIH study sections expect. The increasing time needed by young faculty to establish independent research funding is well documented[8] but not well aligned with the expected rate of transition of COBRE-supported investigators.

Notably, and in our minds regrettably, the challenges we faced have been amplified by changes to the COBRE application requirements. Per the current FOA, "Research Project Leaders must hold a tenure-track faculty appointment (or equivalent at research institutes) at the time the application is submitted." In practice, this can be fulfilled by written departmental confirmation of commitment of faculty-level salary, space and resources independent of the COBRE award. Nonetheless, appointment of non-tenure-track faculty as COBRE Project Leaders creates challenges at institutions like URI and others, which will further constrain the opportunities for such investigators. The period of COBRE support for individual Research Projects has been tightened, which further incentivizes "padding" of COBRE productivity by short-term appointments of investigators already poised to obtain R01 funding. Finally, current FOA requirements require that the lead institution keep most Research Projects and Scientific Cores in a COBRE and limit the total number of Projects and Cores an institution can hold as a subawardee. These changes make a truly balanced collaboration between institutions, such as in our COBRE, extremely difficult. Although these changes have rationale and seek to enhance the productivity and sustainability of COBREs, they may constrain the COBRE mechanism's ability to transform the research landscape.

CONCLUSION

In perhaps a stroke of irony, our Phase I COBRE focused on translational research in infectious diseases immunology closed in early 2020, just as the first wave of the COVID-19 pandemic was cresting in RI. Although we did not succeed in navigating the transition to Phase II, participation in the NIH COBRE program provided a significant direct and indirect financial impact to RI, a valuable learning and growth experience for the senior investigators involved, and incalculable career benefit to several of the young investigators supported.

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Authors

Alan L. Rothman, MD, Institute for Immunology and Informatics and Department of Cell and Molecular Biology, University of Rhode Island.

Jennifer Friedman, MD, Center for International Health Research and Department of Pediatrics, Lifespan, Providence, RI.

Jonathan D. Kurtis, MD, PhD, Center for International Health Research, Lifespan, and Department of Pathology and Laboratory Medicine, Brown University, Providence, RI.

Correspondence

Alan L. Rothman, MD
alan_rothman@uri.edu

COBRE for Skeletal Health and Repair: The Impact of Aging on the Capacity for Peripheral Nerve Regeneration

NEILL Y. LI, MD; JONATHAN GE; BRANDON VORRIUS, MS; EDWARD AKELMAN, MD; QIAN CHEN, PhD

ABSTRACT

Peripheral nerves are crucial to the motor and sensory function provided by our upper and lower extremities to our brain and spinal cord. Following trauma or illness, these nerves may be injured, leading to a loss of function that can be significantly debilitating. Fortunately, given the type of injury and under the right conditions, peripheral nerves can regenerate through well-coordinated biochemical processes. However, as individuals age, the ability for nerves to regenerate becomes less efficient, reducing nerve's potential for the nerve to return to its prior level of function. In this article, we review the research that has been conducted to illustrate the reasons for such a decline in regenerative capacity. In doing so, we explore the concept of inflammaging alongside aging-related impairments of the macrophage and Schwann cell during nerve regeneration.

KEYWORDS: COBRE, bone and joint, aging, nerve regeneration

HISTORY OF THE COBRE FOR SKELETAL HEALTH AND REPAIR

The Center of Biomedical Research Excellence (COBRE) for Skeletal Health and Repair was established in 2007 by National Institutes of Health in Rhode Island Hospital, which is affiliated with the Alpert Medical School of Brown University. It consists of three five-year phases (Phase I: 2007–2012; Phase II: 2012–2017; and Phase III: 2017–2022), which is in its 14th year currently. The COBRE goal is to develop a multi-disciplinary translational research center focusing on discovering mechanisms of cartilage joint diseases and developing prevention and treatment strategies. In the first two phases, seven full project junior investigators received R01 or R01-equivalent federal grants, "graduated" from the COBRE training program, and become leaders in their research fields. They published more than 240 peer-reviewed articles, including landmark discoveries in *Nature*, *Molecular Cell,* and *PNAS*. All 20 pilot-project junior investigators received extramural funding as Principal Investigator. New state-of-the-art laboratories and core facilities have been built in bioengineering, imaging, molecular biology and nanomedicine.

The current Phase III COBRE's main objective is to strengthen and transition the COBRE research infrastructure into a competitive, independent, and self-sustaining academic center of excellence. It consists of an Administrative Core, which provides strong leadership in translational research, evaluates the performance of technical Core Resources and Facilities, guides mentoring efforts in the Pilot Projects Program, and implements the COBRE transitioning plan; the Bioengineering Core, which enhances an interactive research environment and provides the unique resources of biomechanical testing at the cell, tissue, and organ levels; the Imaging, Molecular Biology, and Nanomedicine Core, which enhances translational research from bench to bedside, provides critical expertise and equipment in small animal live imaging analysis, and facilitates development of novel nanomaterial delivery vehicles for diagnostics and therapeutics; and the Pilot Projects Program, which mentors a new generation of researchers in multiple disciplines of musculoskeletal research including clinicians, biologists, and engineers, facilitates research collaborations, and sustains the strong research environment.

The COBRE vision that by sustaining and transitioning the established high-caliber research infrastructure will enable clinicians to work side-by-side with basic research scientists, junior investigators with senior investigators, and biologists with bioengineers for the long term into the future. The COBRE for Skeletal Health and Repair has been recognized as one of the country's premier skeletal research centers by the NIH review panels. It carries out cutting-edge research in injury- and aging-associated degenerative bone and joint diseases and develops tissue repair and regeneration strategies. Peripheral nerve injury and regeneration in the extremities is one of the research areas of the COBRE and the review's focus.

INTRODUCTION TO PERIPHERAL NERVE INJURY AND REGENERATION

Peripheral nerve injuries (PNI) may occur following traumatic mechanisms such as laceration, crush, or stretch injuries resulting from medical conditions such as diabetes, medications, or cancer.[1] In America, over 20 million people suffer from peripheral nerve injuries as a result of trauma and illness.[2] Treatment of these injuries has cost upwards of

1.5 billion dollars, and even after treatment, PNIs may still severely affect patients' quality of life.[1]

Studies have shown that, in general, nerve regeneration occurs about 1 mm per day or an inch a month.[3] The process for regeneration stems from Wallerian degeneration, involving the removal and recycling of axonal and myelin debris distal to the injury site to begin creating an environment favorable for regeneration.[4] This process serves as the innate immune response to nerve damage, and in the peripheral nerve system, primarily involves Schwann cells and macrophages as opposed to oligodendrocytes and microglia in the central nervous system.[5] Wallerian degeneration begins with the death of the damaged nerve to the nearest node of Ranvier via a process called chromatolysis followed by clearing the area of regeneration of axonal and myelin debris.[2] The removal of myelin is a crucial step, as myelin debris contain molecules, such as myelin-associated glycoproteins, that can severely affect Schwann cell migration and regeneration of axons.[5] Both macrophages and Schwann cells have been shown to remove myelin from the environment independently (**Figure 1A**).[5]

Soon after a nerve injury, Schwann cells begin to dedifferentiate and proliferate to aid in clearance and longitudinally realign themselves to form bands of Büngner. The bands of Büngner create a direction and environment that promotes axonal regeneration and remyelination.[4,6] Axonal regeneration starts with forming growth cone at the intact nerve from which axonal sprouting occurs. Through contact guiding and neurotrophic factors, the axons extend until reaching the formed bands of Büngner, where the axons and fascicles are then covered by Schwann cells for myelination (**Figure 1A**).[2]

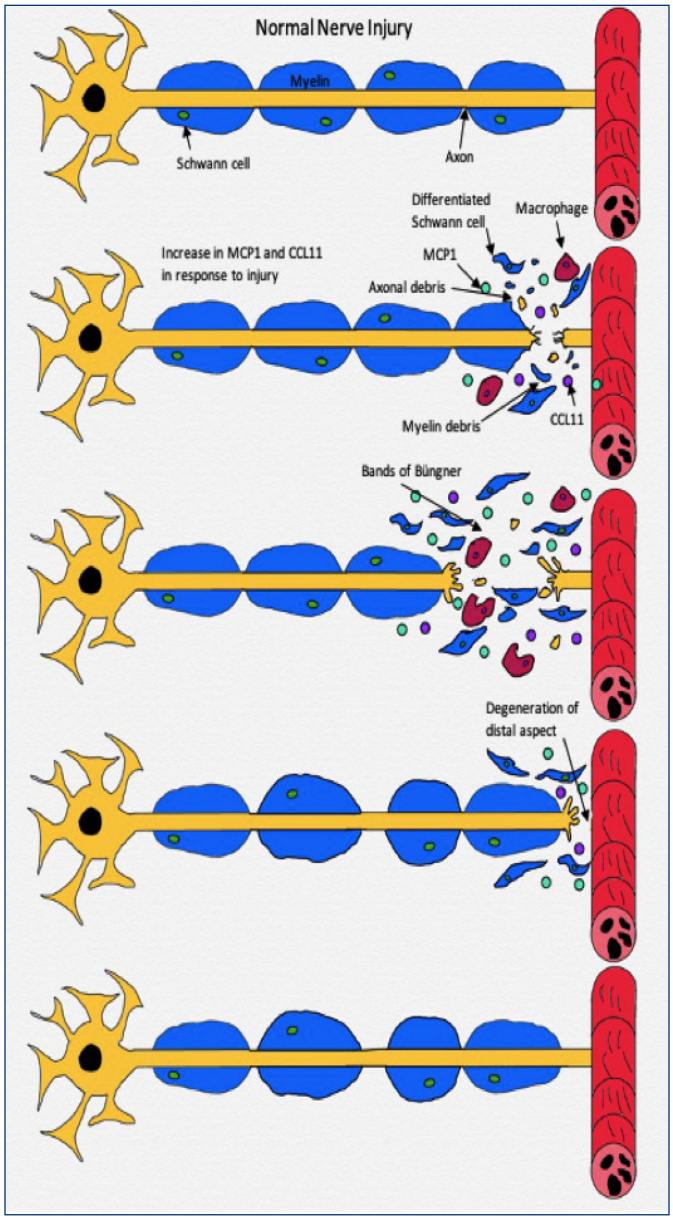
In evaluating this regenerative process's efficiency and effectiveness, studies have found regeneration speeds of peripheral nerves slow with age.[7,8] With age, cells and systemic function become less efficient compared to younger individuals. In addition, with age comes the propensity to develop comorbidities that may affect the health of peripheral nerves due to such conditions as rheumatoid arthritis, Parkinson's diseases, lupus, and Sjogren's syndrome. Peripheral neuropathy may become a symptom of such systemic diseases that may be exacerbated by the decline in the efficiency of nerve regeneration in an aging population.[9,10-12]

The purpose of this review is to evaluate the current understanding of how aging affects peripheral nerve regeneration following injury. In doing so, we explore differences in the microenvironment of nerve regeneration between the young and old by evaluating the inflammatory response to nerve injury alongside the decline macrophages and Schwann cells' function with age.

INFLAMMAGING

As individuals age, their biology becomes less conducive towards nerve regeneration than those who are younger.

Figure 1A. The injury and regenerative process of a nerve in a mature individual. Factors such as MCP1 and CCL11 are upregulated in response to injury. Macrophages are recruited as Schwann cells dedifferentiate to mediate healing. Bands of Büngner formed by Schwann cells then serve to guide regeneration as the distal aspect of the nerve degenerates. Once regeneration is complete, the Schwann cells differentiate to further establish a healthy environment.



One reason this may occur is through inflammaging. Inflammaging is characterized by chronic, low-grade inflammation in the human body that carries risks for poor inflammatory responses when faced with injury or illness.[13] In older individuals, the overall environment is found to be pervasively inflammatory due to an increase in the variety of stressors on the immune system leading to an imbalance between pro- and anti-inflammatory responses.[13]

Inflammatory stimuli that arise from dead cells or debris combined with the declining capacity of the body to clear these materials leads to an autoimmune or autoreactive response that can speed up the aging process in such areas, making them less suitable for regeneration.[13-14] In such inflammatory environments, PNI in aged rats have demonstrated delayed recruitment in macrophages, which subsequently linger at the site of injury longer, producing additional pro-inflammatory cytokines compared to younger rats.[14] While macrophages are typically important for clearing debris, such pro-inflammatory macrophages subsequently suppress Schwann cell function and ultimately axonal regeneration.[15] Rats with crush injuries treated with acetylsalicylic acid (ASA), used as an anti-inflammatory treatment, were found to have accelerated functional recovery, decreased macrophage count, and more advanced remyelination.[14] As a result, the authors suggested that anti-inflammatory treatments may help the progression of nerve regeneration.

Table 1. The role of macrophages and Schwann cells in peripheral nerve injury and regeneration during aging. Intervention targets and treatment options are described.

Components of Regeneration	Intervention Targets	Treatments
Macrophages	• Upregulated CCL1122 29 • Chronically upregulated MCP-1 in a non-injury state8 • Delayed and diminished recruitment to injury site • MCP-1 increase upon injury is downregulated	• ASA has been used as an anti-inflammatory treatment that helps the microenvironment of the regenerating nerve. • Controlled application of cytokines, MCP-1 following injury can improve the progression of regeneration • Inhibition of CCL11 to improve macrophage function
Schwann cells (SC)	• P75, a marker contributing to the differentiation of SCs, is significantly decreased in aged animals, decreasing SC myelination ability • Genetic instability of SCs contributes to the lack of functioning Schwann cell proliferation • c-Jun, a gene responsible for SC proliferation and activity, has its expression impaired in older mice	• Senolytics decrease senescent cell accumulation promoting more effective SC migration to the site of injury 30 • Genetic manipulation to c-Jun and p75 expression could help maintain their function in an aging environment

MACROPHAGE IMPAIRMENT

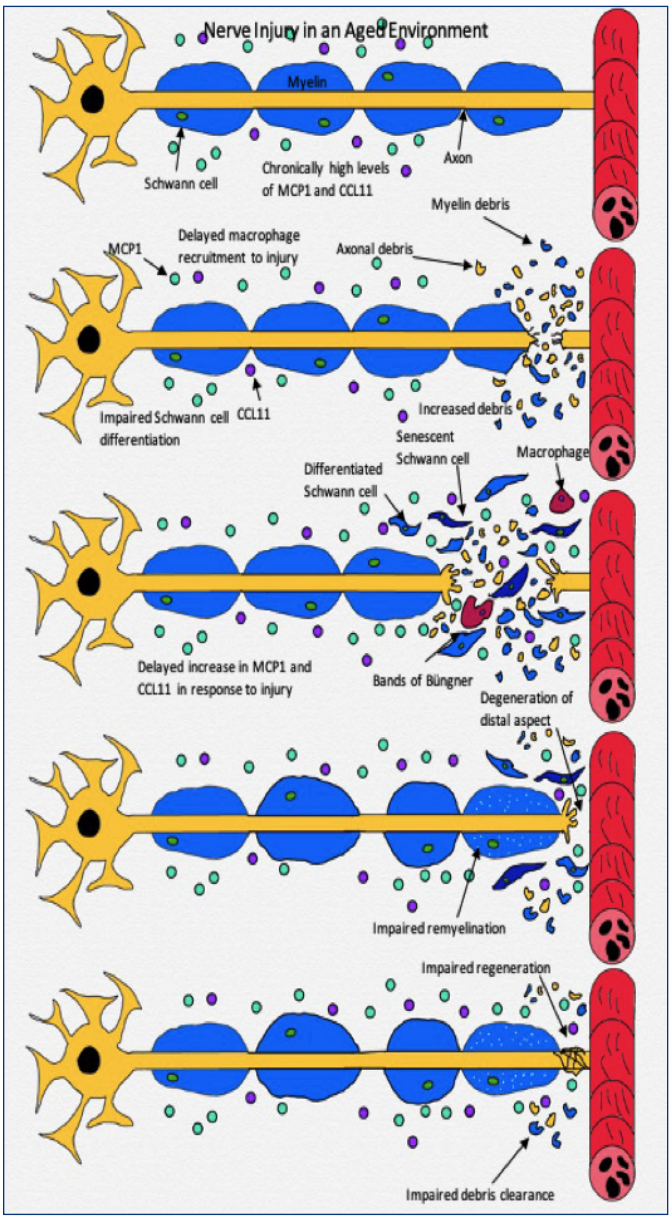
Delayed macrophage recruitment and impairment is a key component behind the slowing of nerve regeneration in aged animals. Following peripheral nerve injury, macrophages help in the clearance of myelin and axonal debris and facilitate angiogenesis and Schwann cell migration to create an environment conducive to regeneration.[8] Given their crucial role, the decline in the recruitment and function of these cells with age has a dire consequence for regenerative ability.

Response to nerve injury is found to be delayed in aged animals due to an accumulation of macrophages later than in young or adult rat models.[14] After about eight days, macrophage count was found to be at comparable levels to younger rats, indicating the magnitude of recruitment may be consistent between ages.[16] Thus, impaired regeneration in aged models could partially be caused by the delay in the recruitment process during the critical early stages of recovery.[16] In addition, studies on age conditioned media (CM) have shown that monocyte migration is significantly less in aged CM than young CM and point towards cytokine signaling of the microenvironment responsible for the delayed recruitment of immune cells.[16] When bone marrow transplants were performed across old and young rats, there was no difference in macrophage counts found across the transplant recipients, indicating that the presence of a young environment or young bone marrow was enough to create a more responsive regenerative environment.[16]

Studies have shown that denervated Schwann cell expression of monocyte chemoattractant protein-1 (MCP-1) plays a significant role in assessing the chemotactic changes of the aged microenvironment.[17] MCP-1 is an important chemokine that regulates the recruitment of macrophages to a site of injury and improves axonal growth.[17] These secretions are a significant component of the chemotactic activity by Schwann cells at the injury site and show the importance of macrophage presence at the site of injury (**Table 1**).[17] With age, studies have shown that MCP-1 released immediately after injury is downregulated in aged nerve injury environments (**Figure 1B**).[16,18] The fewer macrophages initially recruited to the site of injury have been shown to correspond to the decrease in MCP-1 in aged animals contributing to the age impaired regeneration capability of peripheral nerves (**Figure 1B**).

Additional study into MCP-1 has shown that in addition to delayed recruitment of macrophages, its expression persists, producing a chronic inflammatory state shown to hinder continued nerve regeneration.[19] With MCP-1, C-C motif chemokine ligand 11 (CCL11) is another protein produced by macrophages that have been shown to be upregulated in aged peripheral nerve injury environments that interfere with Schwann cell remyelination and axonal regeneration (**Figure 1B**).[14,20-21] While the same macrophage-associated ligands are secreted in peripheral nerve injury environments, the upregulated levels and the persistence they are

Figure 1B. The injury and recovery process of a nerve in an aged individual. With age, MCP1 and CCL11 can become chronically upregulated. In such an environment, macrophage recruitment may be delayed affecting the Wallerian degeneration response to injury. In addition, with age, Schwann cells become impaired in their ability to dedifferentiate causing additional delay in upregulation of regenerative growth factors and an impaired recruitment of macrophages. Some Schwann cells also become senescent and are not active in the regeneration process. Overall, there is reduced clearance of debris following delayed and impaired function of macrophages alongside poor upregulation of a dedifferentiated regenerative phenotype of Schwann cells leading to impaired axonal regeneration and remyelination.



expressed increase with age. These proteins' continued production ultimately leads to macrophage and Schwann cell-related impairment during the nerve regeneration process. Having specifically pinpointed these cytokines, the connection between inflammaging and macrophage impairment in nerve regeneration has become apparent with the potential to act as therapeutic targets.

SCHWANN CELL IMPAIRMENT

Schwann cells (SCs) support peripheral nerves by playing a key role in myelination and axons' remyelination. Upon injury, SCs dedifferentiate into repair cells to secrete regenerative factors, clear debris, recruit macrophages, and lay a path for axonal growth (**Figure 1A**).[8] Like macrophages, SCs also experience negative effects in response to an aging environment They are not as efficient as in younger animals.[8,14,18] As for the cause of a decrease in SC responses, some studies have investigated varying secretion of regenerative factors, genetic changes, and increased damage and fragmentation in the area.

In a study on SCs by Koniyama and Suzuki, the proliferation of SCs throughout Wallerian degeneration in rats of various ages was analyzed.[22] Using thymidine incorporation, the authors were able to measure the proliferation of SCs and fibroblasts. Previous studies have shown that myelin processed by macrophages contribute to SC proliferation during Wallerian degeneration. Koniyama and Suzuki also revealed that axonal components' loss had an inhibitory effect on SCs in actively myelinating nerves.[22] From this data, they were able to determine that the proliferative capacity declined because of both age-related loss of axonal mitogens and a reduction of mitogens from myelin components.

Genetic changes in SCs themselves occur as individuals age, and their functionality also decreases as a result. One example of this is the c-Jun gene (**Table 1**). c-Jun is a critical transcription factor for the presence and proliferation of SCs at injury, and it has been found to vary in levels depending on age.[23] c-Jun is a master regulator in Wallerian degeneration. It controls expression of trophic factors, adhesion molecules, regeneration tracks, and myelin clearance.[24] c-Jun is responsible for the activation of repair mechanisms within SCs by specifying the phenotype of denervated SCs and control over the interactions between axons and SCs.[24] c-Jun presence is induced by injury, as demonstrated by transection of a facial nerve that led to an upregulation of c-Jun during the immune response a few days after injury.[25] In animals without c-Jun, studies discovered the failure of functional recovery, insufficient myelin clearance, failure of axon growth and reinnervation, and death of injured sensory neurons.[25] In a study on aged mice, it was found that aged mice had impaired axonal regeneration while also having a defective cell body response, which lacked both c-Jun expression and phosphorylation.[23] Previous studies have

shown that deletion of the c-Jun gene would cause a delay in recovery and a reduction in innervation, revealing that the regeneration process isn't entirely dependent on the presence of c-Jun.[26] c-Jun has also been known to initiate expression of other regeneration-associated molecules such as CD44, galanin, and α7β1integrin.[25]

As SCs age, there is also the danger of an impairment in the dedifferentiation mechanisms, causing the SCs to remain in a differentiated state. In injury responses, SCs undergo a process of change that alters their structure, molecular profile, and function, creating two distinct differentiation states.[24] p75 is a marker for the repair cell phenotype and has been found to contribute to both dedifferentiation of SCs as well as apoptosis of ineffective and senescent SCs.[27] Studies have shown that animals with a p75 deficiency had significantly impaired motor recovery compared to normal p75 expressing animals through 7–10 weeks.[28] p75 expression is also markedly delayed in SCs in injuries in aged animals when compared to young animals.[8] In aged animals, SCs also exhibit deficiency in myelin clearing ability along with the delayed p75 expression.[8] Inefficient SC differentiation shows a decrease in SC plasticity and SC senescence, which may contribute to the delay in regeneration in the critical stages of injury (**Fig. 1b**). Macrophage recruitment is dependent on factors secreted by dedifferentiated SCs, thus delayed recruitment is one of the downstream effects of inefficient dedifferentiation.[8]

As mentioned previously, macrophage impairment leads to myelin clearance inefficiency. When combined with SCs' impaired ability to clear myelin, the repair response becomes even more inefficient due to age-related changes. It reveals even more downstream effects of the loss of plasticity. Furthermore, because of the plethora of changes SCs undergo with injury responses, they could be especially vulnerable to age-acquired errors in transcription or expression.[8] Studies have shown that older SCs undergo significant karyotype changes when bred in vitro with no anchorage dependency and no increase in telomerase activity: cellular characteristics resembling tumor cells.[29] Overall, genetic instability and genetic changes of aged SCs contributes to the decline of SC function and dedifferentiation capabilities in aged individuals.

DISCUSSION

Nerve regeneration is a coordinated process of cellular dedifferentiation and cellular chemotaxis to generate a microenvironment conducive to healing. In the study of aging as it relates to this regenerative process, a chronic inflammatory state, age-related alterations to the microenvironment, and genetic changes of Schwann cells and macrophages lead to an age-related decline in response to injury and regeneration.

We have reviewed that an aging immune system induces an inflammatory environment called inflammaging, delaying Wallerian degeneration. This inflammatory process also contributes to additional complications associated with delayed macrophage recruitment and impaired macrophage function. Combined with age-related genetic changes leading to poor SC dedifferentiation and SC senescence, nerve injury's response becomes further compromised, negatively affecting the regenerative process.

Potential treatment modalities to improve regeneration in an aged environment involve targeting key cytokines and improving upon cellular function. Chronic elevation of CCL11 with age and its additional expression by macrophages following injury have shown to impair Schwann cell repair activity. Inhibition of this pro-inflammatory target may improve nerve maintenance and repair in the elderly population. In a similar fashion, delayed and subsequent chronic elevation of MCP-1 has been shown to reduce the regenerative support of Schwann cells. Through a timely and controlled application of MCP-1 following injury, axonal regeneration may be improved and better supported.

Genetic impairments of SCs leading to decreased ability of dedifferentiation and increased SC senescence has been demonstrated to be detrimental to nerve regeneration. Potential treatment modalities may involve genetic alterations of aged SCs to increase the expression of c-Jun and the underlying molecular mechanisms for p75 expression to improve the reparative ability of SCs. In addition, to combat against the development of an increasing senescent cell population and its accumulation, senolytics are a potential option. Senolytics are drugs that target and kill senescent cell populations, which present a promising solution to clear senescent cell populations and help restore SC activity and nerve regeneration in aged individuals.

Through a continued understanding of the fundamental aspects of how aging affects regenerative nerve processes, we may be better able to develop therapies to improve upon the response following injury. Suitable interventions will require continued mechanistic studies to improve the localization and targeting of such treatments and further ensure their safety and effectiveness in a vulnerable patient population.

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Authors

Neill Y. Li, MD, Department of Orthopaedics, Alpert Medical School of Brown University, Rhode Island Hospital, Providence, RI.

Jonathan Ge, Department of Orthopaedics, Alpert Medical School of Brown University; Rhode Island Hospital; Program in Liberal Medical Education, Brown University, Providence, RI.

Brandon Vorrius, MS, Department of Orthopaedics, Alpert Medical School of Brown University; Rhode Island Hospital; Center and Graduate Program in Biomedical Engineering, Brown University, Providence, RI.

Edward Akelman, MD, Department of Orthopaedics, Alpert Medical School of Brown University, Rhode Island Hospital, Providence, RI.

Qian Chen, PhD, Department of Orthopaedics, Alpert Medical School of Brown University; Rhode Island Hospital; Center and Graduate Program in Biomedical Engineering, Brown University, Providence, RI.

Correspondence

Qian Chen, PhD
Suite 402A, 1 Hoppin Street
Providence, RI 02903
Qian_Chen@Brown.edu

Our Arduous Research Journey from Preeclampsia to Alzheimer's Disease – Report from the Center of Biomedical Research Excellence (COBRE) for Reproductive Health

SURENDRA SHARMA, MD, PhD

ABSTRACT

This article is contributed by the COBRE for Reproductive Health. The programmatic and scientific goals of this COBRE support a multidisciplinary, translational, and innovative program in women's reproductive health. The research projects focus on using pre-clinical and human models to understand mechanisms of preeclampsia, gestational diabetes, preterm birth, IVF pregnancies, and the application of contemporary computational approaches to identify the networks and pathways underlying these devastating pregnancy complications. We discuss how novel observations emanating from the preeclampsia project can be leveraged to understand chronic diseases such as Alzheimer's disease (AD). Proteinopathy is a hallmark feature of neurodegenerative disorders such as AD. We recently reported that preeclampsia (PE), a severe pregnancy complication, is another prevalent proteinopathy disorder in a younger population. This review provides a comprehensive discussion on shared etiology between PE and AD, establishing a novel blood test for their prediction and diagnosis, and a novel therapeutic option for these disorders.

KEYWORDS: preeclampsia, Alzheimer's disease, proteinopathy, autophagy, blood test

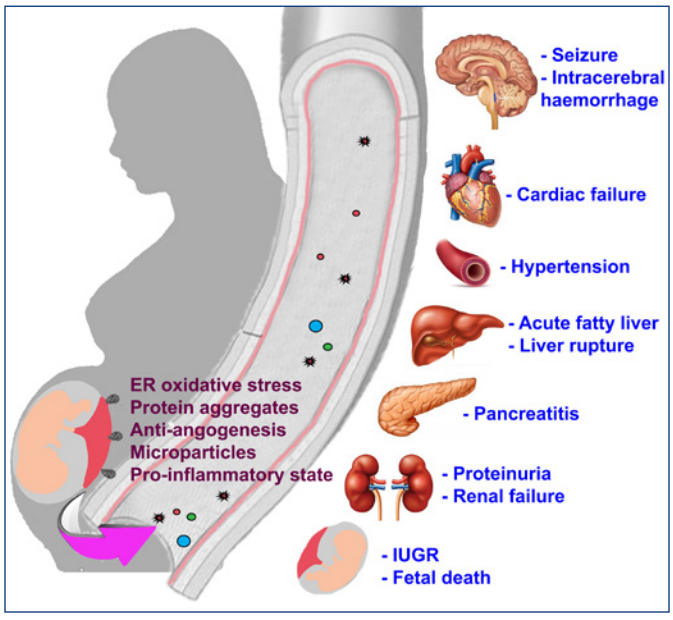
INTRODUCTION

Diseases rarely manifest in isolation. Instead, most are part of a more complex pathway or "pattern" of connected conditions via underlying biological mechanisms. One common denominator in chronic and complex diseases is the role of inflammation and protein mimicry (misfolding and aggregation), which have gained recognition in several human diseases, including Alzheimer's disease (AD).[1-3] Symptoms may emerge across the life course, and large longitudinal databases may help recognize such patterns. Although pregnancy represents a modest portion of the life course, it is now recognized as a window into a woman's future health. It often unmasks predispositions to conditions that only become symptomatic decades later. Preeclampsia (PE) is a pregnancy complication that entails both maternal and offspring health consequences.[4] Recent observations suggest that there is an epidemiological connection between PE and AD.[1,2,5-7] Below, we will discuss the biological and clinical aspects of these devastating disorders and provide details that may lead to their early detection and treatment.

PREECLAMPSIA

Preeclampsia (PE) is a severe pregnancy complication with many manifestations for both mother and offspring. It is a multi-factorial and multi-organ pregnancy complication (**Figure 1**) that affects 3–8% of all pregnant women. It is diagnosed by *de novo* onset of hypertension and proteinuria at or after 20 weeks of gestation.[1-3,8-10] PE can be diagnosed as an early (<34 weeks gestation) or late (>34 weeks gestation) onset severe complication as well as post-partum.[11-13] This devastating pregnancy complication is a placenta-specific disease. In normal pregnancy, the placenta develops in a highly choreographed biological environment programmed by post-implantation cross-talk between the developing placenta and the maternal immune system in the decidualized endometrium. The placenta develops a trophoblast layer comprised of inner villous cytotrophoblasts and

Figure 1. Multi-factorial and multi-organ schematic description of preeclampsia.



multi-nucleated syncytiotrophoblasts. A subpopulation of cytotrophoblasts further differentiate into invading trophoblasts while syncytiotrophoblasts remain in direct contact with maternal blood and act as a storage hub. A subgroup of invading trophoblasts acquires endovascular properties and migrates into constricted endometrial spiral arteries to remodel them into dilated, resistance-free vessels. These resistance-free arteries allow the free flow of nutrients and blood products from the mother into the intervillous space. After that, nutrients and oxygen can cross the syncytiotrophoblast layer into fetal capillaries inside each villous structure. It has been shown that PE is associated with defective spiral artery remodeling. This creates the onset of local ischemia/hypoxia, oxidative stress, and dysregulated immunity at the maternal-fetal interface. The result is a production of an inflammatory milieu and pathological placental nanoparticles (exosomes) containing misfolded proteins, which are released into the maternal circulation.[14] Most importantly, we recently demonstrated that PE is a disease of proteinopathy (e.g., pathologic protein aggregation).[1,2] We have reported that serum from PE patients can induce PE-like features in pregnant mice. In contrast, depletion of protein aggregates in serum blocks the onset of such features, including elevated blood pressure, proteinuria, glomerular endotheliosis, and fetal growth restriction.[1] In this regard, the question arises on what precipitating events lead to the accumulation of protein aggregates in the placenta. We recently demonstrated that the PE placenta is associated with impaired autophagy.[15,16] Autophagy is intricate cellular machinery to maintain homeostasis by its ability to clear cells of misfolded, aggregated protein structures and damaged organelles.[15,16] We proposed that impaired autophagy allows the accumulation of misfolded, aggregated proteins in the PE placenta, causing trophoblast cell death, low differentiation into invading trophoblasts, and defective spiral artery remodeling.[15] Moreover, we have shown that PE is associated with gasdermin D/caspase 3-mediated sterile inflammation in the placenta, a possible trigger for the onset of protein aggregation and trophoblast cell death.[3] We anticipate that impaired autophagy and protein aggregation can be targeted for therapeutic intervention in PE.

ALZHEIMER'S AND RELATED NEURODEGENERATIVE DISEASES

The pathological hallmark of AD and its related neurodegenerative diseases is the accumulation of hyperphosphorylated tau as intracellular tangles and amyloid-β as extracellular plaques in AD and its prodromal condition, mild cognitive impairment (MCI).[17,18] α-Synuclein accumulates as aggregated protein in Lewy bodies in AD and Parkinson's disease, and other distinct proteins in other neurodegenerative diseases.[19] Like PE, protein misfolding, aggregation, and impaired autophagy are also intertwined in AD. Tau can manifest in diverse isoforms stemming from distinct phosphorylation patterns and sites, imbalanced isomerization involving trans and cis configurations, and the preponderance of C-terminal microtubule-binding peptide region.[20-24] Tau aggregates without amyloid-β involvement are also present in frontotemporal dementia and corticobasal degeneration. Although pathological protein misfolding and aggregation in neurodegenerative diseases have been accepted for a long time now, it has been difficult to leverage these findings for prediction or therapeutic intervention. To date, no well-defined, cost-effective, non-invasive blood test has been developed to diagnose AD. The widely used tests currently rely on cerebrospinal fluid protein analysis and position emission tomography (PET) imaging, which are invasive and expensive.[25,26] Recently, efforts have focused on blood tests for AD and MCI. However, they still depend on the identification of a single, non-aggregated protein with pre-evaluation manipulation of plasma samples.

DO THE SAME PROTEINS UNDERGO AGGREGATION IN PE, AD, AND MCI?

An important question that is often asked is whether we have access to pathological markers or a blood test that can enable diagnosis in much larger populations at a pre-AD age. Similarly, this argument can also be made for PE. We took advantage of our observations of impaired autophagy and protein aggregation in PE. We hypothesized that autophagy-deficient trophoblasts would not clear protein aggregates, and the accumulated aggregated structures can then be identified by an immunofluorescence probe. We have established an autophagy deficient human extravillous trophoblast cell line by stably transfecting a mutant autophagy gene that blocks the assembly of autophagosomes and reduces lysosomal expression machinery proteins. Our novel blood test for detecting serum protein aggregates depends on the exposure of autophagy-deficient trophoblasts to serum for 12–24 hours, fixing the cells, and then staining with an immunofluorescence dye for estimation of total protein aggregates.[27] With specific antibodies, we can co-localize and identify individual proteins. Our data suggest that transthyretin,

Figure 2. Identification of protein components of serum aggregates from patients with preeclampsia, Alzheimer's disease, and mild cognitive impairment.

Preeclampsia	Alzheimer's Disease	Mild Cognitive Impairment
Transthyretin	Transthyretin	Transthyretin
Amyloid-β	Amyloid-β	Amyloid-β
Tau231	Tau231	Tau231
Cis P-tau	Cis P-tau	Cis P-tau
—	α-Synuclein	α-Synuclein

amyloid-β, and hyperphosphorylated tau are common in the protein aggregates among PE, AD, and MCI. In contrast, the protein aggregates in AD, and MCI also contain α-synuclein (**Figure 2**).[27] This suggests that PE and AD share etiological biomarkers, and these observations may suggest a common therapeutic option(s) for both disorders.

CAN IMPAIRED AUTOPHAGY AND PROTEIN AGGREGATION BE USED AS THERAPEUTIC TARGETS FOR PE AND AD?: COMMON THERAPEUTIC OPTIONS FOR PE AND AD

To date, no effective therapy is clinically available for PE. The most effective treatment is the delivery of the placenta and fetus. PE is accompanied by severe health consequences not only during pregnancy but also in later life. PE patients are at higher risk of developing cardiovascular disease, diabetes, and possibly AD as suggested by our lab and that of others.[1,2,5-7,14,15] Our group's preliminary data are innovative in their support of a predictive assay for PE and investigation of a small, non-mammalian disaccharide molecule targeting autophagy and proteinopathy. This molecule can reverse the cellular and pathological events associated with PE. Our preclinical model for screening possible therapeutic options is a novel approach that is likely to lead to novel therapeutic options for PE.

Since both PE and AD share impaired autophagy and protein aggregation as key pathological factors, it is fair to predict that a drug that targets these cellular pathways may have therapeutic potential to prevent and/or treat these devastating conditions. We plan a similar therapeutic approach to prevent or treat the onset of AD-like symptoms. The disaccharide drug blocks appearance of AD-like pathology in h-tau AD transgenic mice. In *in vitro* experiments, this drug is quite potent in restoring autophagy and blocking protein aggregation in response to endoplasmic stress inducers such as hypoxia. The drug entails no detrimental effects in non-pregnant, pregnant, or wild-type mice. Notably, the offspring born to disaccharide-treated mothers were of normal weight and showed no ill effects through a few months of their life. As described above, the novel blood test and the disaccharide drug have become the focus of our recent efforts for planning pilot prediction and clinical trials for PE and AD.

CONCLUSIONS

A syndrome of younger age can rarely provide mechanistic and therapeutic insights for devastating chronic diseases such as AD, which entail a huge socio-economic burden on the healthcare systems. Diagnosis and treatment of such conditions have suffered from a lack of appropriate animal models, non-invasive blood tests, and target-based therapies. Thus, it is clinically important that a well-defined treatment modality be pursued that may eventually lead to randomized clinical trials. We discuss here that a pregnancy complication, preeclampsia, share etiological and therapeutic insights with AD and its prodromal MCI condition.

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Author

Surendra Sharma, MD, PhD, Professor of Pediatrics; Director, COBRE for Reproductive Health, Providence, RI.

Correspondence

Surendra Sharma, MD, PhD
ssharma@wihri.org

Building Research Capacity in Vascular Biology in Rhode Island

SHARON ROUNDS, MD; ELIZABETH O. HARRINGTON, PhD; SUSAN F. McNAMARA, MS

ABSTRACT

The CardioPulmonary Vascular Biology Center for Biomedical Research Excellence (CPVB COBRE) was funded in 2013 by the National Institute for General Medical Sciences to establish a collaborative center for research excellence in vascular biology in Rhode Island. The CPVB COBRE has funded successful junior faculty investigators and pilot projects spanning the research spectrum from basic vascular development mechanisms using zebrafish to clinical research on pulmonary hypertension to the effects of mindfulness on hypertension in pregnancy. The Administrative Core has united the group with an active seminar program with visiting experts, a focus on career development, and the use of evaluation to support continuous improvement. The Cell Isolation and Organ Function Core has provided high-quality research services and expertise. Most importantly, hard-working and creative physicians and basic scientist investigators and mentors have worked together to expand the spectrum of vascular biology research in Rhode Island.

KEYWORDS: vascular biology, pulmonary, cardiology, interdisciplinary

INTRODUCTION

Cardiovascular and pulmonary diseases are among the leading causes of morbidity and mortality in the US and the world.[1] Coronary artery disease (CAD) is the leading cause of morbidity and mortality globally, with greater than 8 million deaths in 2019. Chronic obstructive pulmonary disease and lower respiratory diseases are the 3rd and 4th leading causes of death worldwide, respectively, with greater than 5 million deaths in 2019, and are frequently complicated by pulmonary vasculopathy and cardiovascular co-morbidities that markedly worsen prognosis. In 2020, COVID-19, caused by the SARS-CoV-2 virus, has become the 3rd leading cause of death for persons aged 45 through 84 years and the 2nd leading cause of death for those aged 85 years or older, as compared to other leading deaths in 2018.[2] Most patients with COVID-19 die from respiratory failure or vascular complications, including stroke, myocardial infarction, or thromboembolism, and COVID-19 is recognized as a disease directly impacting the endothelium.[3] In addition, deaths due to cardiopulmonary vascular diseases manifest health inequity and are increased in lower socio-economic populations and settings.[1,4] Thus, cardiopulmonary vascular diseases are important causes of human suffering, for which more effective treatments and prevention are needed.

VISION AND PROGRAMS OF THE CPVB COBRE

In 2013, the CardioPulmonary Vascular Biology (CPVB) Center of Biomedical Research Excellence (COBRE) was established through funding from the National Institutes of Health, National Institute of General Medicine and Sciences (NIH, NIGMS) and is currently in Phase 2 of funding. This center's visions are to unite clinical and basic scientific investigators from multiple disciplines and foster research career development of those who have not yet established an independent research program (**Figure 1**). The goals are to enhance understanding of vascular cell injury mechanisms and develop and strengthen an interdisciplinary collaborative research center with strong technical support and career development activities across Rhode Island institutions. The CPVB COBRE has brought together investigators from the Vascular Research Laboratory at Providence VA Medical Center (PVAMC), the Surgical Research Laboratory, Cardiovascular Research Center, Cardiothoracic Surgery Research Laboratory, and the Division of Pulmonary/Critical Care/Sleep Medicine at Rhode Island Hospital (RIH), as well as

Figure 1. Schematic representation of strategies used by the CardioPulmonary Vascular Biology COBRE in accomplishing the vision of the center.

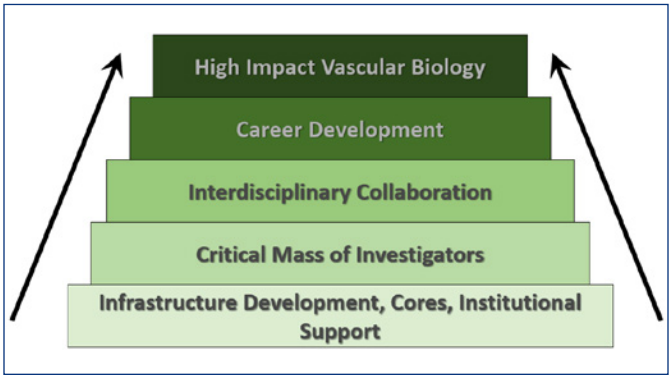
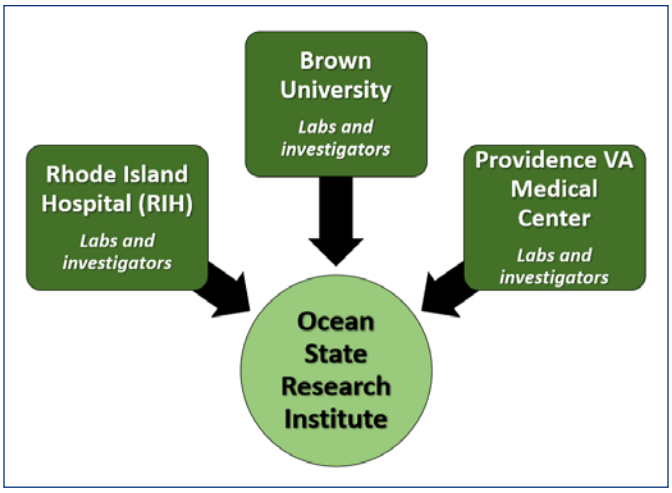


Figure 2. Institutional structure of the CardioPulmonary Vascular Biology COBRE.



from Brown University departments within the Division of Biology & Medicine (**Figure 2**). Investigators are supported as either Project PIs for 2–5 years or as Pilot Project PIs for 1–2 years, with funding prioritized by scientific review in the manner of NIH study sections. A key feature of the CPVB COBRE program is fostering interactions among clinicians and biologists, in the strong belief that interdisciplinary research is most likely to successfully translate discoveries to patient care.

Each CPVB COBRE junior faculty investigator is supported by a mentoring team of experienced senior researchers who provide scientific expertise and guidance, career advice, leadership skills, professional development, organizational advancement, networking opportunities, and examples of work-life balance. In addition, institutional and administrative support from the PVAMC, Ocean State Research Institute (OSRI), RIH, and Brown University has provided an infrastructure for CPVB COBRE investigators, enabling them to focus their efforts on establishing a productive research program.

The CPVB COBRE has two core facilities that are available to all investigators and mentors. These include an Administrative Core and a Cell Isolation/ Organ Function (Cell/ Organ) Core (**Table 1**). The Administrative Core facilitates the research activities and career development of junior investigators by providing fiscal assistance, organizing career development opportunities, managing mentorship committee meetings, organizing meetings with our external and internal advisory committees and other activities, as described in Table 1. The Cell Isolation and Organ Function Core is a scientific core that focuses on cultured cells and whole organ structure and function to investigate vascular injury and repair. The Cell/Organ Core provides a unique skill set and expertise to Rhode Island vascular biologists by providing high-quality cell isolation, characterization, and propagation of vascular-derived cells; tissue morphometry and image analysis; and heart and lung function. The Cell/ Organ Core services are described in Table 1. A new scientific core to assess Respiratory Function is in the planning stages.

A vigorous program of research seminars provided by visiting speakers and local experts is a critical aspect of the CPVB COBRE program. The CPVB COBRE sponsors twice-monthly seminars. Each project and pilot project investigator presents results of "work-in-progress" and research and professional achievements at least twice per year. In addition, the CPVB COBRE seminar series is complemented by the monthly Brown Investigators in Respiratory Diseases (BIRDS) lecture series and (in 2020) the "Decoding COVID" and "Decoding Disparities" seminar series, sponsored by the Division of Biology and Medicine. These seminars feature both local experts and visiting speakers. Junior faculty investigators serve as the primary host for guest lecturers, providing them with an opportunity to become acquainted with the distinguished visiting researcher, thereby increasing their professional network. Indeed, these visits have led to scientific collaborations for some junior investigators. To

Table 1. Services provided by CPVB COBRE Cores

Core	Service
Administrative	Junior Investigator fiscal assistance
	Organize and manage career development opportunities
	Managing mentorship committee meetings
	Manage meetings with our internal and external advisory and executive committees
	Maintains the cpvb.org website
	Provides financial management of entire program
	Tracks investigator outcomes
	Manages evaluations of the mentor-mentee teams
	Assesses scientific core efficiency and program effectiveness
	Manages seminar series and guest lecturers
	Manages intramural and extramural progress reports
Cell Isolation & Organ Function	Provides high quality cell isolation, characterization, propagation and functional assessment of vascular-derived cells; including endothelial cells from heart, lung, and other organs, cardiomyocytes, fibroblasts, and epithelial cells
	Provides tissue morphometry and image analysis
	Assessment of heart, lung, vessel structure and function and angiogenesis
	Transient gene manipulation in vivo
	Acquire, establish, and disseminate technologies and instrumentation to provide state of the art research tools for vascular biology research in Rhode Island
	Establish processes and procedures that support the sustainability of the Cell/Organ Core

leverage resources, the CPVB COBRE has co-sponsored lectures with other research seminar series at Brown, including the Pathobiology; Molecular Pharmacology and Physiology; and Grand Rounds in Cardiology, Pulmonary, and Internal Medicine. With funding partners, the CPVB COBRE and BIRDS seminar series have sponsored 70 visiting speakers from 29 universities since 2013.

In 2020, the CPVB COBRE Principal Investigators (PIs) partnered with the University of Mississippi Medical Center Cardiorenal and Metabolic Disease Research Center COBRE and the University of Louisville COBRE in Diabetes and Obesity Research PIs to establish a monthly multi-COBRE seminar series. This seminar series features research talks by COBRE investigators and brings together investigators from related disciplines to build a network of collaborations among the COBRE programs.

Finally, the CPVB COBRE has contributed significantly, both administratively and financially, to the Rhode Island IDeA annual symposium since 2015. At these day-long symposia, the junior investigator from each of the Rhode Island INBRE or COBRE programs present their work in short talk or poster format. Thus, the CPVB COBRE has regularly scheduled opportunities to highlight the junior investigator's work both within and outside of Rhode Island, fostering professional growth and career development.

A key element to the CPVB COBRE program and junior investigator successes has been the engagement of the advisory committees, including the executive committee (EC), an internal advisory committee (IAC), and an external advisory committee (EAC). Both the IAC and EAC meet twice annually, while the EC meets every other month. For each meeting, the PIs provide updates, including successes and challenges to the program. While the EC meetings discuss financial expenditures, junior investigator issues, and current and immediate future goals, the IAC and EAC meetings discuss the program's overarching vision. In addition to meeting with the CPVB COBRE PIs, the EAC meets with each junior investigator and mentoring team to discuss achievements, hurdles, and next steps. In these private meetings, the EAC members provide frank feedback to the junior investigators, to help them achieve research independence. The dedication, consistent participation, and wise advice provided by each of the committees have been crucial in the program's ongoing success and junior investigators.

OUTCOMES OF CPVB COBRE

Since 2013 the CPVB COBRE has supported 10 junior faculty investigators from 5 academic departments and 18 pilot project investigators with 24 distinct pilot projects. In addition, the COBRE has supported two administrative personnel and 65 research assistants. Thus, the COBRE has substantially impacted vascular biology research in Rhode Island.

The Administrative Core of the CPVB COBRE supports on-going quantitative and qualitative program evaluation. More than half (6/10) junior faculty project investigators have achieved RO1 funding, and 5/18 pilot project Investigators have achieved RO1 funding since 2014. CPVB COBRE Junior faculty investigators have also been awarded substantial research support from foundations, such as the Falk Foundation and the Harold S. Geneen Foundation, and other federal sources, such as the Departments of Defense and Veterans Affairs CPVB COBRE investigators have contributed to the knowledge of vascular biology by publishing 83 publications with CPVB COBRE support. In keeping with our goal of enhancing scientific collaboration, 12 investigators supported by the CPVB COBRE have published manuscripts documenting collaborations with other CPVB investigators and/or mentors.

The CPVB COBRE strongly espouses continuing quality improvement and has therefore used survey methodology to assess program effectiveness. In collaboration with Judy Kimberly, Evaluation Director of the Brown University Division of Biology and Medicine, survey instruments were developed for junior faculty investigators and mentors. The semi-annual surveys address investigator self-efficacy, research mentor interactions, and effectiveness of core support activities. Although generally the program has been considered effective, there continue to be areas for improvement. The content and results of these surveys have been published in the Journal of Clinical and Translational Science.[5]

CONCLUSIONS

The CardioPulmonary Vascular Biology COBRE was established in 2013 to develop interdisciplinary research in lung, heart, and vascular diseases in Rhode Island. Critical elements of success include institutional support, interdisciplinary collaborations, a focus on career development and networking, a vigorous program of visiting researchers, and on-going program evaluation. The most important element of success has been the expertise of outstanding, hard-working, and collaborative junior faculty researchers and mentors with whom the CPVB COBRE has been privileged to work.

After 7.5 years of funding, there has been substantial progress toward the ultimate goal of improving outcomes of vascular diseases in Rhode Island. Continued challenges include maintaining a "pipeline" of junior faculty engaged in vascular biology research, sustaining research in difficult pandemic times, and growing sustainability of scientific cores.

Acknowledgments

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Authors

Sharon Rounds, MD, Vascular Research Laboratory, Providence VA Medical Center, Providence, RI; Department of Medicine, The Warren Alpert Medical School of Brown University, Providence, RI.

Elizabeth O. Harrington, PhD, Vascular Research Laboratory, Providence VA Medical Center, Providence, RI; Department of Medicine, The Warren Alpert Medical School of Brown University, Providence, RI.

Susan F. McNamara, MS, Ocean State Research Institute, Providence, RI.

Correspondence

Sharon Rounds, MD

Providence VA Medical Center

Research 151

830 Chalkstone Avenue

Providence, RI 02908

Sharon_rounds@brown.edu

www.cpvb.org

COBRE for Computational Biology of Human Disease at Brown University: Progress and Prospects

DAVID M. RAND, PhD; ASHOK RAGAVENDRAN, PhD

ABSTRACT

We provide a program update on the COBRE Center for the Computational Biology of Human Disease (CBHD) at Brown University and affiliated hospitals. High throughput data from multiple 'omics-level' technologies are fundamental factors in identifying and treating human disease. The acquisition of these data is now straightforward, but the efficient and creative interpretation of these data remains a serious impediment to progress for faculty at all levels in both the basic and translational aspects of biomedical science. The CBHD COBRE seeks to build close collaboration between laboratory scientists working with model systems and data scientists working with computational and bioinformatics tools that can accelerate human disease research implementation. We describe the accomplishments of junior faculty Project Leaders (9) and Pilots Project leaders (8) and the objectives of the CBHD COBRE's core facility: The Computational Biology Core (CBC). To extend the CBHD COBRE's reach in the future, we encourage one and all to visit the CBHD COBRE and bring your data sets and questions. Only by engaging with new people and challenges can the program grow to serve the broader biomedical research community in the State of Rhode Island.

KEYWORDS: bioinformatics, data science, genomics, reproducibility

INTRODUCTION

Discovery in the biomedical sciences today requires expertise in computational biology and bioinformatics. The diversification of genome-enabled technologies has created a data deluge that can only be rendered useful by accelerating computational and bioinformatic innovation. Notable examples are the discoveries emerging from the Pan Cancer Analysis of Whole Genomes[1], the Genome Aggregation Database (gnomAD)[2] and the ENCODE3 release.[3] These massive efforts by teams of computational, laboratory, and clinical scientists are defining the molecular and bioinformatic landscapes for new research in the biomedical sciences. All researchers will benefit from accessing, engaging, and contributing to these resources through their research programs. For junior faculty in early stages of their independent careers, or more senior researchers employing wet-lab or clinical approaches, realizing the full benefits of these computational resources can be a significant challenge.

This reciprocal relationship between biomedical advances and computational sophistication demands that scientists pursuing the basic biology of human disease must have a working knowledge of the effective application of computational and bioinformatic resources. When the revolution in molecular biology began in the 1980s, disparate biology fields were unified by a common language of DNA cloning, hybridization, expression, and creative manipulation. The boundaries between biochemistry, cell biology, evolution, and genetics were rendered permeable by methods that allowed researchers to ask common questions. The computational revolution has the same effect today but on a broader scale. Researchers across all the STEM fields are making significant contributions to computational biology with relevance to human disease's basic biology. The engineering advances allowing long-read sequencing, or single-cell transcriptomics have the needs for data structures enabling efficient processing of massive sequence files, which has spurred statistical geneticists to develop novel analysis tools. These, in turn, allow clinicians and wet-bench molecular biologists to pursue entirely new questions about human disease.

This context is the motivation for the COBRE for the Computational Biology of Human Disease (CBHD). To keep pace with biomedical progress, all researchers need to build 'omics-enabled' computational approaches into the ecosystem of their research program, and to be successful, most people will need support for the development of these skills. In many institutions, bioinformatic analyses are commonly provided in a core facility that performs analyses for biomedical researchers. In this kind of service role, the science behind the computational and algorithmic analyses may be 'outsourced' by the biomedical researcher, reducing the need for those individuals to develop skills in these areas. The CBHD COBRE seeks to extend this service model by building a community where the biomedical researcher and the computational biologist are part of the same team and work together to design, execute, analyze, and interpret genome-enabled inquiry as a unified workflow. The CBHD COBRE aims to help researchers from complementary disciplines build advanced computational and bioinformatics approaches into collaborative research programs. To

make this effort thrive, the COBRE seeks to assemble a diverse community of biomedical researchers whose individual questions may differ widely, but whose underlying analytical questions share lots of common ground. By building a community where these approaches and ideas can cross-fertilize, junior faculty will not only reach independence more rapidly, but faculty at all levels will be better equipped to advance the later stages of their careers by managing the data deluges that will undoubtedly emerge in the future.

Most COBRE programs invest in a Core Facility that provides critical equipment that individual researchers cannot acquire independently. The CBHD COBRE has devoted all its core facility resources to hiring data scientists and building a Computational Biology Core (CBC) facility. The four CBC staff members have the necessary computational or bioinformatic expertise to support junior faculty Project Leaders (PLs). Through collaboration with Brown University's Advance Research Computing group, they eventually provide this service to the broader biomedical community in Rhode Island. This approach has filled a much-needed gap in the research environment at Brown leading to multiple successful NIH and NSF awards to the junior faculty Project Leaders. In the first five years of the CBHD COBRE program, we have learned how to support for a wide range of researchers from molecular biologists with no computational skills to statistical geneticists seeking help with software engineering. Based on these learned experiences, in the next phase of the CBHD COBRE we seek to extend and strengthen the Center with support for a new cohort of Project Leaders and increased support for the CBC staffing that will broaden the reach of the CBHD COBRE beyond COBRE investigators. The COBRE

Table 1. Grant support to junior faculty members who have been supported by the CBHD COBRE. The top half of the table lists the initial Project Leaders, the bottom half lists Pilot awardees, four of whom have transitioned to Project Leader status (Belenky, Crawford, Ene, Wood), and one who is a Project Leader in this Phase 2 proposal (Beura). Names in red are Project Leaders awarded R01/R35 support and have or will graduate. The total costs listed are for the duration of the grants awarded.

Project Leader	Grant	Institute	Project Title	Dates	Total Costs
Phase 1 Initial Project Leaders					
Jamieson	R01	NIHLB	Influence Of The Lung Microbiome On Macrophage Responses To Lung Damage	4/18–3/23	$2,437,369
Neretti	R01	NIA	The Role Of Somatic Transposition In Age-Associated Genomic Instability	9/17–8/22	$2,176,898
Ramachandran	R01	NIGMS	Novel Statistical Methods To Localize Genomic Elements Underlying Adaptive Evolution	2/17–1/21	$1,607,269
	T32	NIGMS	Predoctoral Training Program In Biological Data Science At Brown University	7/18–6/23	$942,600
Vaishnava	R01	NIDDK	Role Of Epithelial Cell Intrinsic Vitamin A Metabolism in Regulating Immune Function in The Gut	7/18–6/23	$2,256,411
Phase 1 Pilot Awardees					
Belenky	R21	NCCIH	Dietary Fiber To Mitigate Antibiotic-Induced Microbiome Dysbiosis: A Multi-Omics Approach	7/18–6/20	$487,500
	R01	NIDDK	Relating Impacts of Antibiotics on the Gut Metabolome and Microbiome to Host Physiology and Weight	9/20–8/25	$2,536,655
	AFRI	USDA	Mitigating antibiotic-induced microbiome disruption with whole grains	6/20–5/23	$500,000
Beura	Searle Scholar		Adaptation of Resident Memory CD8 T Lymphocytes in the Reproductive Mucosa	7/20–6/23	$300,000
Crawford	Sloan Fellowship		Deep Learning in the Discovery of Gene Interactions in Disease	9/19–8/21	$75,000
	R35 MIRA	NIGMS	Interpretable Machine Learning for Characterizing Broad-sense Heritability in Complex Traits and Rare Diseases	9/20–8/25	$1,869,560
Ene	R21	NIAID	Defining The Roles Of Perseverance And Heteroresistance In Persistent Human Fungal Infections	6/18–5/20	$446,875
Webb	R01	NIA	Molecular Mechanisms Underlying The Preservation Of Neural Stem Cell Quiescence During Aging	7/17–6/22	$2,269,826
Wood	R03	NIA	Transcriptomic and epigenomic landscape of neurodegenerative disease models	1/21–12/22	$318,000
Total					**$18,223,963**
Total: R01s and R35 only					**$15,153,988**
Initial COBRE Grant					**$11,500,000**

welcomes new requests for analyses of 'omics scale data sets, experimental designs, and software engineering to be successful. Only though the increased engagement with the larger community can we hope to reach the goal of sustainable computational biology support for the broader biomedical community at Brown University, its affiliated hospital research centers, and all researchers in Rhode Island.

PROGRESS

Research grants from junior faculty Project Leaders (PLs)

The CBHD COBRE has provided support for five initial PLs, six replacement PLs and eight Pilot Awardees in Phase 1. The PLs and Pilot awardees have successfully obtained external funding, with six R01s and an R35 MIRA awarded to date. Four of these awards were to the initial PLs and the remaining three were to Pilot awardees, two of whom transitioned to PLs after initial PLs graduated with R01 funding. Other notable grant successes are a T32, a USDA grant, two R21s, an R03, Searle Scholar Award and a Sloan Research Fellowship (see **Table 1**).

Publications and Presentations

The Project Leaders and Pilot awardees have been productive in publishing during Phase 1 of this COBRE. **Table 2** lists the number of peer-reviewed publications by each COBRE investigator, whether a Project Leader or Pilot awardee. A similar activity is apparent in the number of conference presentations by these Project Leaders, or students in their laboratories.

Table 2. Peer-reviewed publications and conference presentations by CBHD PLs and Pilot Awardees.

Project Leader	Year started	Peer-reviewed Publications	Conference Presentations
Belenky	2017	17	10
Beura	2020	0	0
Crawford	2018	8	26
DeCecco	2019	5	0
Ene	2018	5	4
Huang	2019	5	0
Jamieson	2016	12	29
Lisi	2019	5	8
Neretti	2016	16	22
Ramachandran	2016	15	36
Singh	2020	0	0
Uzun	2016	12	21
Vaishnava	2016	6	8
Webb	2017	9	11
Wood	2019	0	3
Total		**115**	**178**

Transitions of junior investigators

The Phase 1 project included five initial junior faculty PLs. Four of these PLs have received independent R01-level funding and graduated from COBRE support. The CBHD Pilot Program funded two one-year awards each year for four of the five years, supporting eight Pilot Awardees. As the initial PLs graduated, Pilot Awardees applied to fill these positions, ultimately supporting 11 PLs. Seven PLs have graduated from the CBHD COBRE program during Phase 1. Of the eight Pilot awardees, five have been recruited to full Project Leader status with a sixth Pilot Awardee being recruited as a PL in Phase 2.

Build the Computational Biology Core (CBC)

An important accomplishment of the Phase 1 project, and an ongoing goal of the CBHD COBRE, is to build and maintain the CBC. Based on surveys of analysis needs across the Brown biomedical community, three requests emerged: 1) to provide analysis and computing support for large-scale biomedical datasets for COBRE PLs; 2) to create and maintain a shared data repository and interfaces for projects within the COBRE and for additional projects in the local research community; and 3) to serve as a locus of formal and informal training, discussion and exchange of ideas on computational and statistical issues related to human disease (See **Figure 1A**). Hiring staff in this area is challenging given the competitive market for computational skills in the biotechnology industry. Four PhD-level data scientists staff the Computational Biology Core, and efforts are in place to continue the COBRE support to ensure this level of expertise can be maintained. The primary responsibility of the CBC staff is to support the COBRE PLs and Pilot Project awardees. To date this has included a wide range of activities from data analysis (RNA-seq, microbial 16S analysis as metagenomics, variant calling, CutandRun analyses for mapping DNA binding, and single-cell RNAseq) to software engineering (new software tool or expanding the application of a PL's in-house development). This work with COBRE PLs has led to joint publications in both biomedical discoveries and software development.[4,5]

To expand CBC's reach to the broader Rhode Island biomedical community outside the COBRE PLs, the CBC staff have been developing reproducible bioinformatic workflows that are freely available and built-in Docker or Conda environments that increase portability to different operating systems ranging from personal laptops to Linux clusters. The focus has been on developing an infrastructure for long-term sustainability following best practices for: a) reproducibility in analysis, b) software engineering processes and procedures for building tools, and c) documentation (See **Figure 1B**). The tools for running standard bioinformatics workflows and streamlined storage of reference data are available at the CBC website (cbc.brown.edu). In addition, the CBC has partnered with other research centers at Brown, such as the

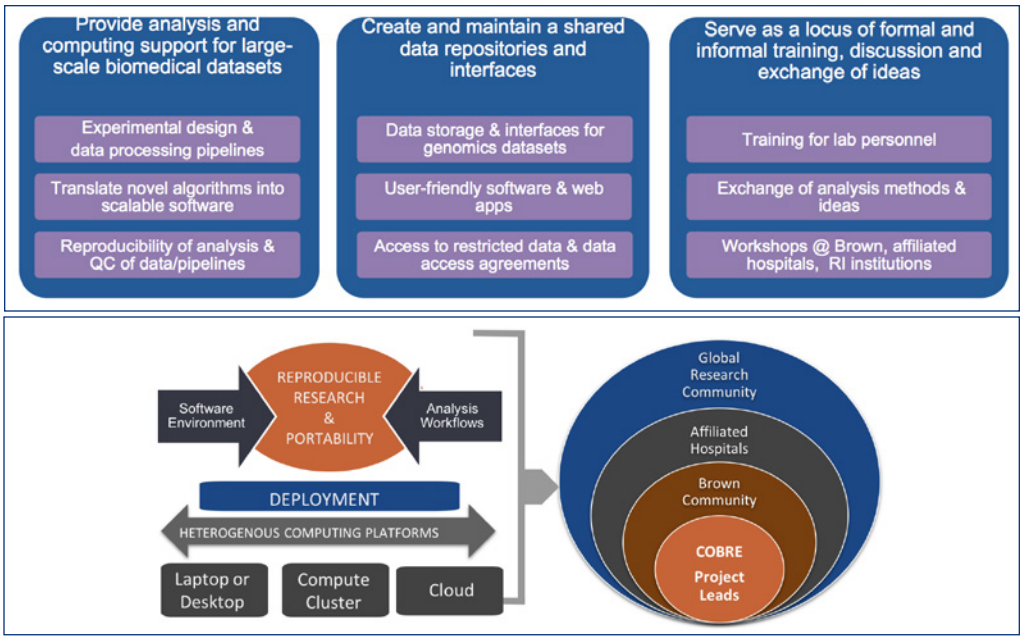


Figure 1. Objective of the Computational Biology Core (CBC) of the CBHD COBRE.

A. (top) Three primary objectives of the CBC.

B. (bottom) Approach for enhancing reproducible data analysis by investigators and increasing portability of analyses to multiple operating systems. Efforts in these areas start with COBRE Project Leaders, and grow out to the broader community.

Brown Center for Biomedical Informatics (BCBI), the Center for Computational Molecular Biology (CCMB), and Brown's central computing operation the Center for Computation and Visualization (CCV), to develop standardized procedures for the transfer and storage of large and restricted data sets (e.g., Globus, dbGaP). An advantage of devoting a COBRE Core facility to human resources (four data scientists) rather than specific pieces of equipment, is that equipment can become obsolete, but engaged data scientists can continue to learn. Clearly, both kinds of resources require continued support to remain a cutting-edge service facility. The main approach the CBHD COBRE is taking in pursuit of this goal is by learning from the broader community and integrating with other Centers and RI IDeA programs.

Outreach, training and workshops for the broader community

The CBHD COBRE and CBC's long-term goal is to serve as a hub for training by offering workshops and other forms of outreach to the broader Brown biomedical community. This has been realized through workshops on several bioinformatics workflows, such as RNAseq, microbiome analyses, and Ingenuity Pathway Analysis (IPA), among others. An important component of this outreach is the Open Office Hours program. Any user from the Rhode Island biomedical community can receive guidance on computational or bioinformatics analyses (the schedule for making an appointment is on the CBC website: cbc.brown.edu/services/#consulting). The CBC has had more than 228 participants in workshops from 59 departments (including 8 clinical), 41 participants in Office Hours from 17 departments, 86 participants in the IPA sessions, and 49 general consulting sessions (see **Table 3** and **Figure 2**). These outreach activities provide service

Table 3. Numbers of individuals from the Brown biomedical community engaged in various services provided by the Computational Biology Core (CBC).

CBC Service	Workshops	Office Hours	IPA	Consulting
Unique Users	228	41	86	49
Departments	59	17	28	14
Faculty	34	9	35	13
Grad Students	94	17	26	17
Postdocs	44	7	6	11
Undergrads	21	2	5	4
Other Staff	35	6	14	4
Total	**228**	**41**	**86**	**49**

Figure 2. Distribution of visits to different components of the Computational Biology Core's (CBC) workshops, office hours and training sessions, available to all members of the Brown biomedical community.

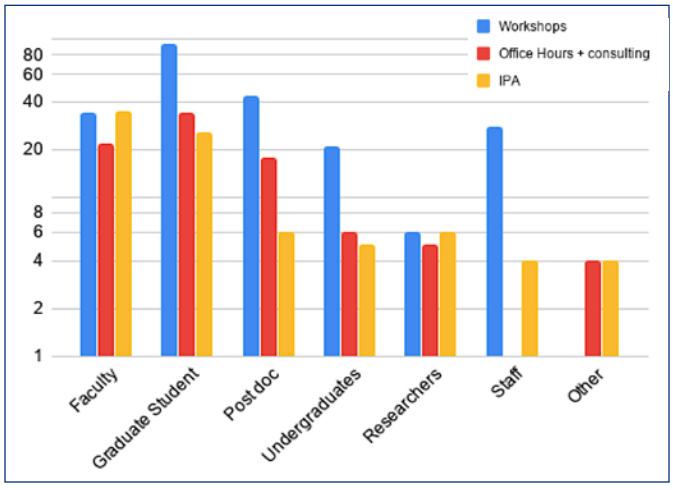
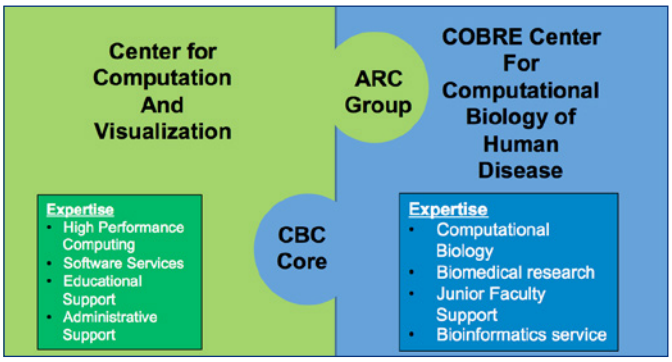


Figure 3. Collaborative relationship between the CBC and the ARC within the larger CCV and CBHD COBRE environments.



to the broader community and provide a picture of future needs for sustainability. The diversity of departments that have participated indicates there is a broad user base for future analyses. It has led to plans for collaborative efforts to seek external funding to support additional CBC staff in the future.

Accommodating this growing base of researchers has been greatly facilitated by the collaborative relationship between the CBHD COBRE and the Advanced Research Computing (ARC) group that resides within Brown's Center for Computation and Visualization (CCV). The ARC group provides computing services for other units at Brown outside the biomedical sciences, offering depth in computational, statistical and informatics expertise that can strengthen the domain-specific skill sets behind computational biology *sensu stricto*. This affiliation creates an integrative working environment where new approaches are freely shared enabling creative solutions to researchers' needs (see **Figure 3**). Extracting useful information from high-throughput technologies have blurred the boundaries between traditional topics in biostatistics (e.g., experimental design, multi-hypothesis testing), applied mathematics (e.g., graph and network theory, diffusion processes), and computer science (efficient algorithms, software engineering, database management, machine learning, etc.). This affiliation between the four CBC data scientists and 15 ARC staff creates an integrative working environment where new approaches are freely shared. By working in a diverse, supportive ecosystem, the team will learn more and stay current with best practices.

PROSPECTS

Lessons learned

The first five years of the CBHD COBRE have provided lessons in how to achieve sustainability into the future. A key factor is to adapt to changing service needs as the COBRE investigators graduate and are replaced, and as other members of the RI biomedical community seek assistance with their research. The pace of change in genomic and computational technologies has only accelerated in recent years, as has the breadth of researchers needing access to these technologies in the Rhode Island biomedical community. Microbiome analyses were in high demand among our initial cohort of PLs, and this has been replaced with single-cell RNAseq and various methods for quantifying chromatin accessibility. Single cell transcriptomics was an emerging technology a few years ago but has become a routine application. Notably, there are at least 15 different computational analysis pipelines for interpreting these data.[6] Moreover, machine learning has exploded across all of data science with deep and wide applications in biology and medicine. Thus, the 'computational biology of human disease' covers a wide range of topics from wet-bench molecular biology to 'omics'-scale data sets, to software engineering. Sustaining excellence in this area for the State of Rhode Island can only be achieved by integrating with the other Centers, Institutes and IDeA programs across the State.

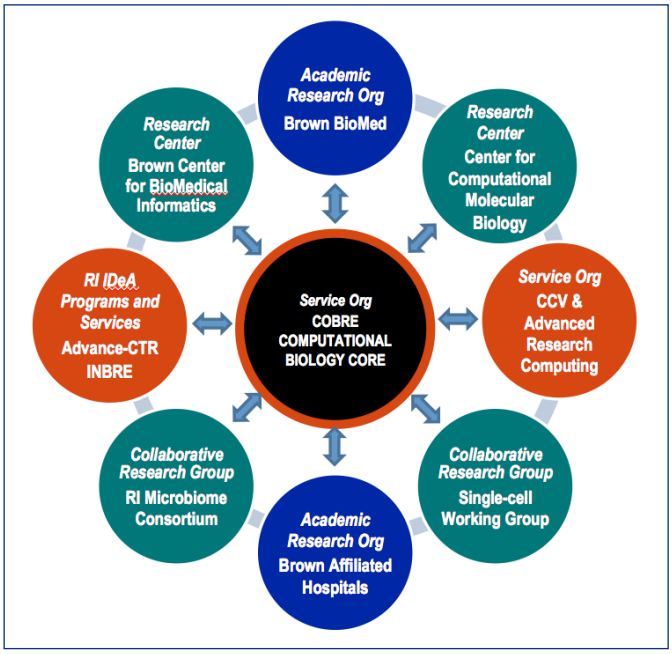
Integration with Centers, Institutes, and IDeA Programs

All of the IDeA programs in Rhode Island, by definition, strive to enhance biomedical research excellence in the State. The focus on medical and translational concerns is explicit in some programs (e.g., Advance-CTR), but research on non-human model organisms can take center stage in other programs. An important goal for the breadth of IDeA programs in RI is to maintain this support by coordinating resources across programs to avoid redundancies and enhance collaboration. The CBHD COBRE initiated a coordinating meeting with of Core Facilities Managers for all IDeA program in December 2019 to share goals, identify possible overlaps and collaborations, and ensure that resources are complementary. While all IDeA program had Design and Analysis Cores to meet each program's specific needs, there was little overlap among the various Core facilities in immediate goals or expertise. From the perspective of computational and bioinformatic analyses of omics-scale biomedical data, the CBHD COBRE's Core (CBC) remains highly complementary to other IDeA Program Core facilities, as well as other research entities at Brown, the affiliated hospitals and URI. The RI-INBRE supports several Core facilities including a dedicated PhD staff person in Bioinformatics (Dr. Chris Hemme). The relationship between the RI-INBRE Bioinformatics Core and the CBHD COBRE is highly collaborative and complementary, especially in microbiome research. Dr. Hemme has worked with COBRE PL graduate Dr. Peter Belenky at Brown to initiate the Rhode Island Microbiome Consortium, hosting a Symposium in late 2019. In addition to being at different institutions, the demand for bioinformatics work through the RI-INBRE network is such that there is no overlap in our COBRE CBC services and the RI-INBRE bioinformatics services. This kind of collaboration is what will increase capacity and sustainability in the future.

In seeking to build the strength of biomedical research excellence in RI, we will continue to maintain

communications among these programs and ensure that the resources developed by each program can build the network of excellence that the IDeA program intends. The relationships among these entities is illustrated in **Figure 4**. In conclusion, and looking to the future, the simplest thing a researcher in Rhode Island can do is to come visit the CBHD COBRE and bring your data sets and questions. Only by engaging with new people and challenges can the program grow to serve the broader biomedical research community in Rhode Island.

Figure 4. Relationships between the COBRE Computational Biology Core (CBC) and other academic and service units at Brown, its affiliated hospitals and other IDeA programs in Rhode Island. The CBC is the only entity offering genome-enabled computational services to the Brown biomedical community. CCV is the Center for Computation and Visualization.



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Authors

David M. Rand, PhD, Department of Ecology and Evolutionary Biology and Center for Computational Molecular Biology, Brown University, Providence, RI.

Ashok Ragavendran, PhD, Department of Ecology and Evolutionary Biology and Center for Computational Molecular Biology, Brown University, Providence, RI.

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The views expressed herein are those of the authors and do not necessarily reflect the views of NIH.

Correspondence

David M. Rand, PhD

Department of Ecology and Evolutionary Biology and Center for Computational Molecular Biology

Brown University, Providence Rhode Island 02912

401-863-2890

David_Rand@brown.edu