BASR
2023

Biomedical Primate Research Centre
Annual Scientific Report 2023

Welcome

Join our journey through health research and alternatives

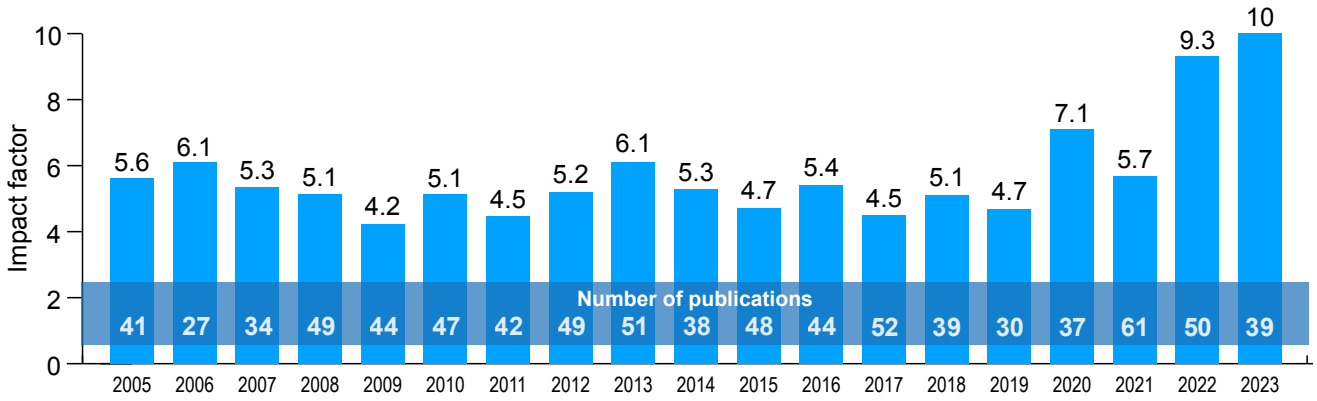
Biomedical Primate Research Centre

Message from the director

For BPRC and its employees, the year 2023 was, seen from a scientific point of view, very productive. We made significant advances in our research on infectious diseases such as malaria, TB, denque, MERS but also the post-COVID syndrome.

The same holds true for our efforts on improving animal welfare and the genetic characterization of the immune system with the aim to achieve disease model refinement. As can be seen, the different departments have summarized their major achievement (see the research area section of this annual report) and as usual a comprehensive overview is listed on the news item section on our website.

Altogether, in 2023 we published 39 manuscripts in the peer reviewed scientific literature with an average impact factor of 10. Which is a new record and highlights the quality of our research output. This high impact factor is due to the fact that a substantial segment of our research was published in leading scientific journals.

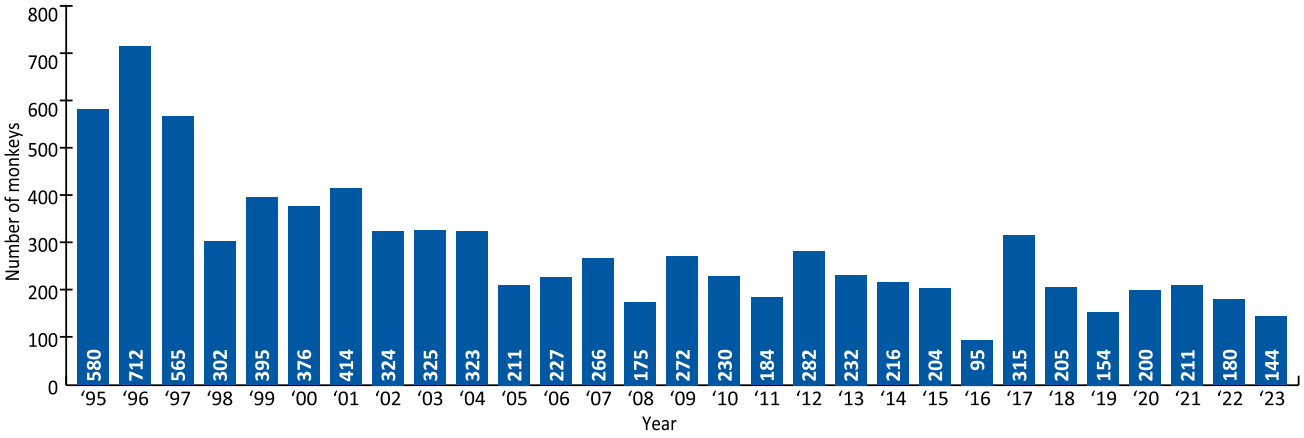


Our staff members also attended many conferences and actively displayed their results for a wide scientific audience. A special event was that our director, Ronald Bontrop, delivered the highly esteemed Ceppellini-award lecture during the EFI-conference in Nantes.

Message from the board

Also 2023 was a financially sound year and we closed the books with a small negative result of of 6 K€. We made a lot of investments which will allow us to improve our research even more in the future. Even better some of these investments will boost our non-invasive types of research.

In 2023 our colony measures approximately a 1000 animals. We have conducted on an annual basis 144 experiments in our animal preclinical models of human health and disease. The results that were obtained will be published soon in the peer reviewed literature.



Prof. dr. R.E. Bontrop
Director of BPRC

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Monkey Research

- Explained -

Welcome to the 2023 annual scientific report from Biomedical Primate Research Centre (BPRC). In this report our scientists inform you about their work with monkeys and their most important scientific findings. As you will see, our work covers many different aspects, collaborations with (inter)national partners and (inter)national funding agencies. Together, this highlights our work as high standard and scientifically relevant.

On January 1st, 2023, BPRC housed 944 monkeys, 617 rhesus macaques (*Macaca mulatta*), 230 cynomolgus monkeys (long-tailed macaques (*Macaca fascicularis*) and 97 common marmosets (*Callithrix jacchus*). On December 31st, 2023, BPRC housed 968 animals, 677 rhesus macaques, 203 long-tailed macaques and 88 common marmosets.
In 2023 BPRC worked with 144 animals, 117 rhesus macaques, 22 long-tailed macaques and 5 common marmosets. These numbers were reported to the NVWA.

BPRC is committed to health research and alternatives. The development and implementation of the 3Rs, **R**efinement, **R**eduction and **R**eplacement are visible throughout BPRC. In this report you will find many examples of how refinement of animal models leads to a reduction of the number of animals we work with.

Why do we still need animals for research?

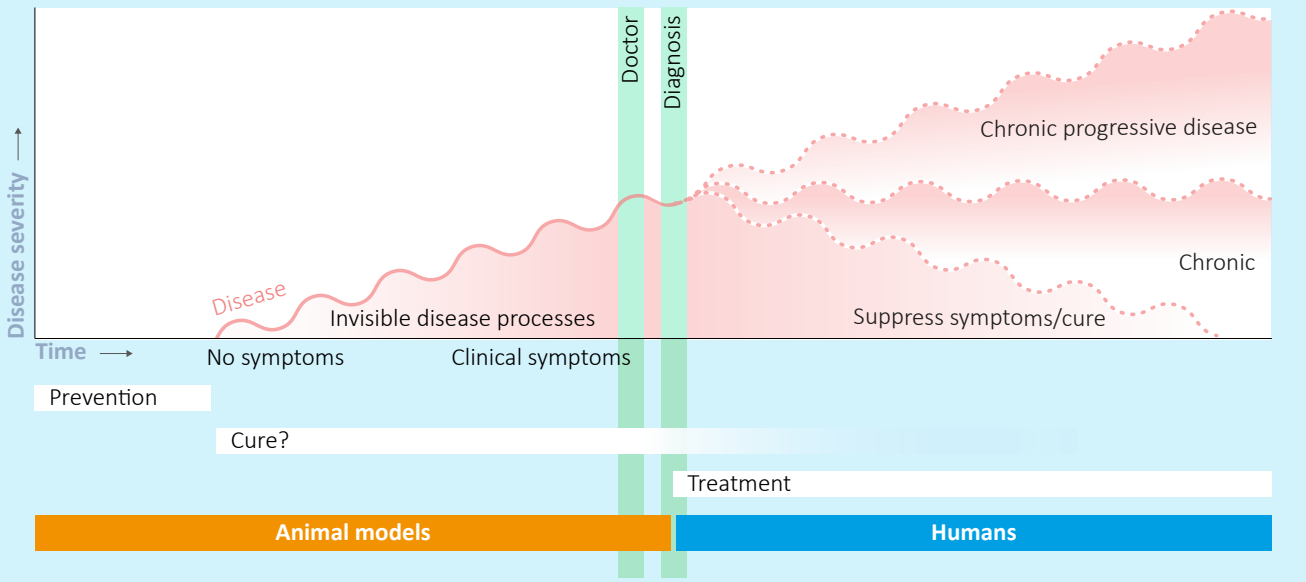
BPRC focuses on life threatening and/or debilitating diseases that affect millions of people. Diseases without cure or treatment because the complicated disease mechanisms are not yet fully understood. The European Commission concluded that the type of research conducted at BPRC cannot be done without life animals.

Visualizing invisible disease processes

A patient only seeks medical help when he or she is suffering from disease symptoms. At that time the actual disease-process is already ongoing and caused damage to cells and/or organs. As a consequence, early and asymptomatic stages of a disease cannot be studied in people. To ‘visualize invisible’ disease processes we depend on experimental animal models.

Monkey Research

- Explained -



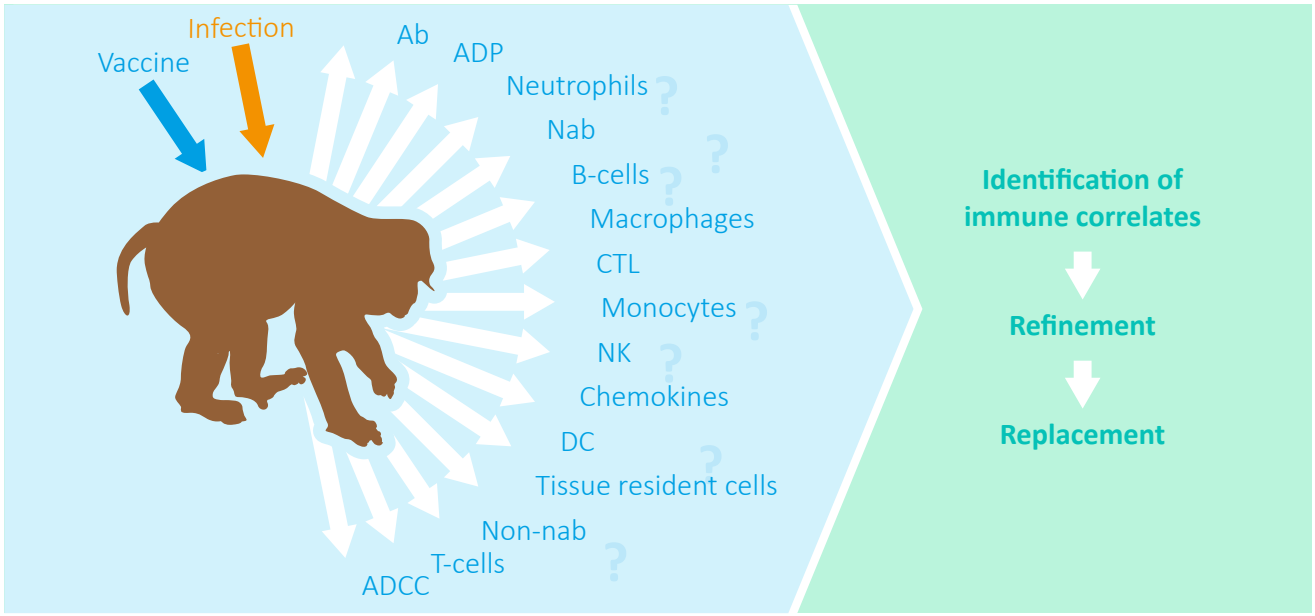
The X-axis represents the time and y-axis disease severity. A disease does typically not start with clinical symptoms. The onset is often without signs. But as time progresses the damage to cells and organs accumulate and cause clinically relevant disease. Depending on the disease this can take days to years. At that point patients go to a doctor and laboratory tests are needed to make a diagnosis. Only when diagnosis is made, proper treatment can start. Most diseases have a so-called point of no return. Before that the damage can be repaired, but when damage progresses beyond that point it results in irreversible (unrepairable) damage.
In best case, the disease is diagnosed before the point of no return. Damage caused by the disease is reversible and treatment cures the disease. In case of a chronic disease, the disease cannot be cured. Drugs can help to suppress further disease progression but the damage is irreversible and drugs cannot undo the damage (MS). During a chronic progressive disease, the symptoms are also irreversible and get worse. So far there are no drugs available to stop progression. Some infectious diseases are preventable by a vaccine (measles) or prophylactic drugs (malaria). Studying a disease and potential new medicines in people is only possible after the diagnosis of the disease. To study early events, we rely on animal models that resemble the infection or disease in humans. Understanding early events of a disease enables the identifying of the point of no return, hence decrease overall medical health care costs and increase quality of life. But also, to develop animal-free alternative methods to evaluate new potential medicines.

Creating conditions for animal free alternatives

Unraveling disease processes is not only necessary to identify potential treatments but also to create the conditions for animal free-alternative methods to test vaccines or new treatments. Before one can even think about the development of an animal-free method to evaluate potential drugs or treatment, one needs full understanding of a disease and its critical events.

Monkey Research

- Explained -



In a prophylactic vaccine study, a vaccine is used to generate a pathogen-specific immune response. The interplay between thousands of different molecules, including antibodies, cytokines, specific subsets of cells in the blood and chemokines, determine the quality and quantity of the immune response, and thus the protective effect of the vaccine. To test this, the animal is exposed the actual pathogen. The protective capacity of the vaccine is defined by the amount of virus, bacteria or parasite that can be detected after exposure. Little or no pathogen means the vaccine was successful.

Identification of (a combination of) molecule(s) that predict the effectiveness of the vaccine on forehand is a powerful refinement of an animal model. In the first place because evaluation of future new vaccine candidates does no longer require exposure to the pathogen itself to determine the effectiveness of a vaccine and therefore the discomfort of the animal is reduced. And second because it is the first step to the development of animal-free alternative techniques to evaluate potential new vaccine candidates.

Vaccine efficacy studies

Vaccines are a safe way to generate immune memory without the potential risk to develop disease-associated complications. Many infectious diseases can be prevented by vaccines, but for many pathogens vaccines are desperately needed. To evaluate the efficacy an experimental vaccine so called exposure studies are required. After vaccination the immune response is challenged by the actual pathogen. This requires a model that is susceptible to vaccination and the pathogen. This makes rodents often not the best model.

Monkey Research

- Explained -

As most vaccines evaluated at BPRC are developed for human use, people would be the best model. However, only for a limited number of infectious diseases human challenge studies are permitted, like malaria and influenza. To limit medical risk for the human volunteers, these human exposure studies are typically performed with weak, attenuated or curable strains of the pathogens, and only with vaccine candidates that have proven safety in animal models. Yet for the vast majority of the vaccine efficacy studies conducted at BPRC human challenge models are not available.

In this report we proudly present our contribution to science, the 3Rs and the development of animal-free alternatives.

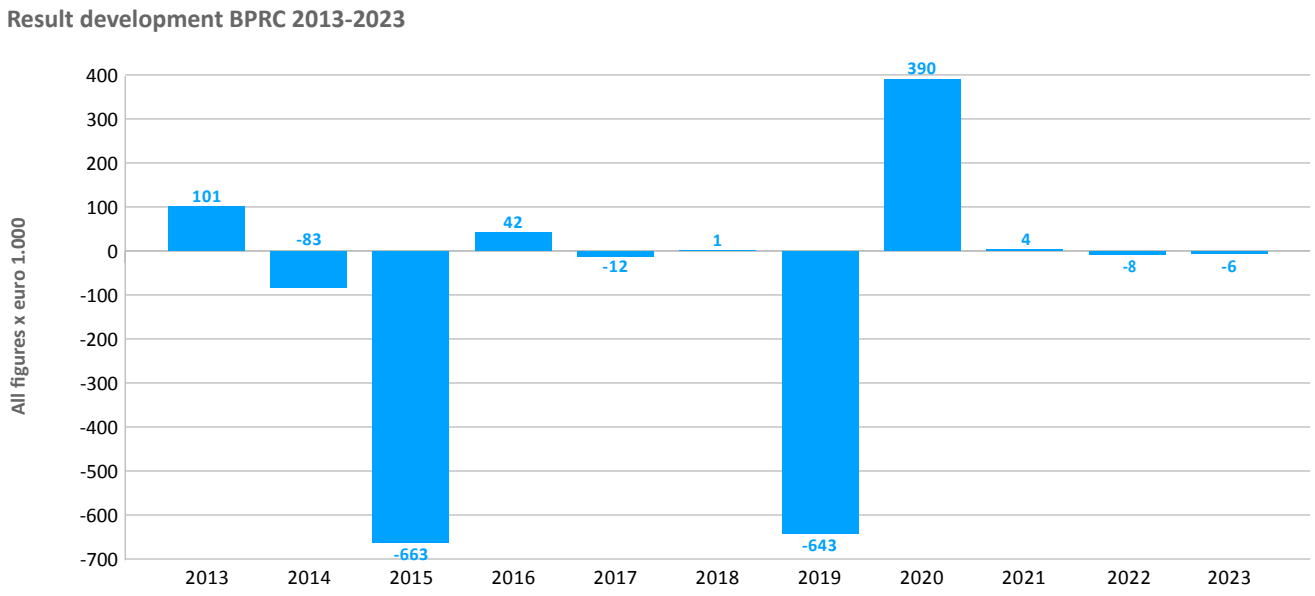
External links:

More information regarding animals in experiments is found at Stichting Informatie Dierproeven, European regulatory bodies (Directive 2010/63/EU), the Dutch law (Wet op de dierproeven). The Centrale Commissie Dierproeven (CCD) is the legal body in The Netherlands that is authorized to provide licenses. BPRC's accreditation by AAALAC International guarantees good institutional policies, animal husbandry and welfare, veterinary care at BPRC.

Our Financial Results

The Foundation Biomedical Primate Research Centre (BPRC) closed the fiscal year 2023 with a small negative result of 6 K€.

The total turnover of projects increased from 13,2 million euros in 2022 to 14,2 million euros in 2023. This increase in income is due to more income which are covering costs. The operational costs are 0,1 percent lower than the budget for the year 2023.



Our Financial Results

	2023 (K€)	2022 (K€)
Turnover projects (extern)	4.221	4.280
Turnover projects (subsidy)	9.720	8.747
Total turnover projects	13.941	13.027
Other excluding interest	237	211
	237	211
Total turnover	14.178	13.238
External direct project costs	478	346
Staff costs	8.566	8.095
Depreciation	715	589
Other operating charges	4.471	4.177
Total operating costs	14.230	13.207
Profit/loss on ordinary activities	52-	31
Interest	46	39-
Profit for the financial year	6-	8-
Tax	-	-
Profit for the financial year after tax	6-	8-

EFFECTIVE PERSONNEL

	2023		2022	
Service Departments	16,4	17%	16,3	17%
Animal Sience Department	40,0	40%	43,5	44%
Research	42,7	43%	38,9	39%
Total	99,1	100%	98,7	100%

	2023 (K€)		2022 (K€)	
ASSETS				
FIXED ASSETS				
Buildings and structures	22.537		24.106	
Tangible fixed assets	1.354		1.306	
		23.891		25.412
CURRENT ASSETS				
STOCKS		89		65
DEBTORS DUE WITHIN ONE YEAR				
Work in progress	632		3.179	
Receivables from contracts	449		620	
Receivables tax	428		39	
Other receivables	239		196	
		1.748		4.034
Cash at bank and in hand		13.237		11.975
Total assets		38.965		41.486

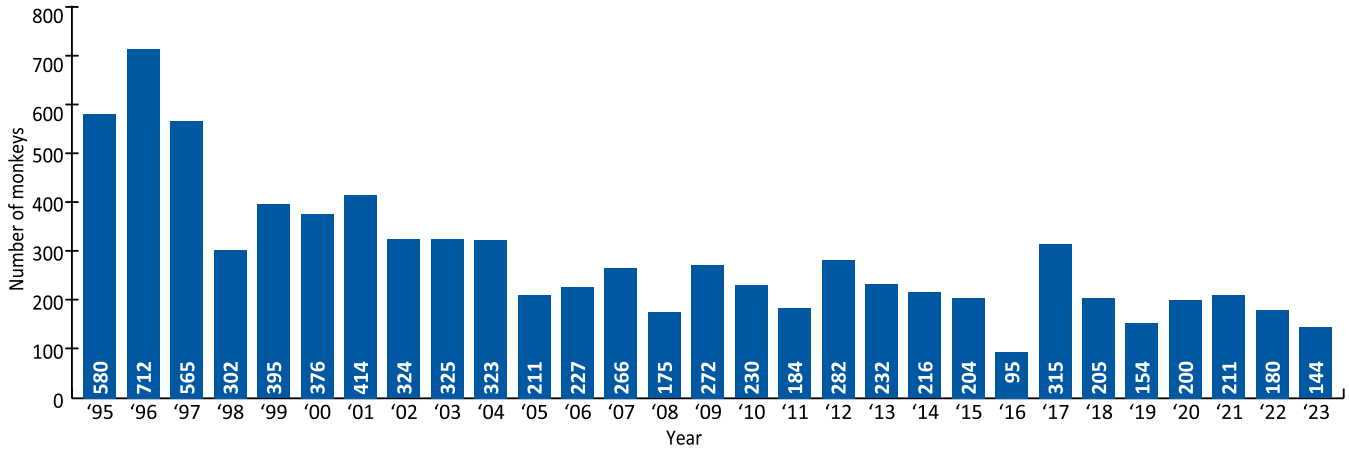
	2023 (K€)		2023 (K€)	
LIABILITIES				
EQUITY				
Equity	4.504		4.513	
Revaluation reserve buildings	4.351		4.869	
Result current year	6-		8-	
		8.849		9.374
PROVISIONS				
Primates	-		-	
Deferred tax liabilities	-		-	
(Flexibel) retirement	-		-	
Repairs buidings	1.662		1.381	
		1.662		1.381
LONG TERM DEBTS				
Bank	16.648		17.904	
Received in advance on asstes	5.626		5.901	
		22.274		23.805
SHORT TERM DEBTS				
Received in advance on projects	1.723		2.637	
Received in advance on assets	251		311	
Received in advance subsidy	700		740	
Accounts Payable (TAX)	423		404	
(Flexibel) retirement	114		104	
Accounts Payable	443		620	
Commitment Bank	1.256		1.195	
Other liabilities	1.270		915	
		6.180		6.926
Total liabilities		38.965		41.486

Our Financial Results

WNT-Verantwoording Organisatie BPRC	
BEZOLDIGING TOPFUNCTIONARISSEN	
Leidinggevende topfunctionarissen	
Gegevens 2023	
Bedragen X 1€	
Functie(s)	Directeur
Aanvang en einde functievervulling in 2023	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	172.714
Beloningen betaalbaar op termijn	33.593
Subtotaal	206.307
Individueel toepasselijk bezoldigingsmaximum	223.000
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT
Bezoldiging	206.307
Reden waarom de overschrijding al dan niet is toegestaan	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT
Gegevens 2022	
Aanvang en einde functievervulling in 2022	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	166.174
Beloningen betaalbaar op termijn	28.886
Subtotaal	195.060
Individueel toepasselijk bezoldigingsmaximum	216.000
Bezoldiging	195.060

Toezichthoudende topfucntionarissen				
Gegevens 2023				
Bedragen X 1€				
Functie(s)	Lid Raad van Toezicht (Voorzitter)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2023	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging				
Bezoldiging	13.988	NVT	10.000	10.000
Individueel toepasselijk bezoldigingsmaximum	33.450	NVT	22.300	22.300
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT	NVT	NVT	NVT
Reden waarom de overschrijding al dan niet is toegestaan	NVT	NVT	NVT	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT	NVT	NVT	NVT
Gegevens 2022				
Aanvang en einde functievervulling in 2022	1/7 - 31/12	1/1 - 30/06	1/12 - 31/12	1/1 - 31/12
Bezoldiging	4.639	3.328	0	6.959
Individueel toepasselijk bezoldigingsmaximum	16.200	10.800	1.800	21.600
Gegevens 2023				
Bedragen X 1€				
Functie(s)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2023	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging				
Bezoldiging	10.000	10.000	10.000	10.000
Individueel toepasselijk bezoldigingsmaximum	22.300	22.300	22.300	22.300
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT	NVT	NVT	NVT
Reden waarom de overschrijding al dan niet is toegestaan	NVT	NVT	NVT	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT	NVT	NVT	NVT

Facts & figures



The number of monkeys we worked with over the years and the scientific achievements in 2023.

A total of

39 scientific publications

in peer reviewed international journals.
Complete list

In addition we

train 6 PhD students

to write and defend their thesis.

We placed

36 updates on our website

in the newsfeed.

Health, Safety & Environment

Health and Safety of our colonies and our staff as well as the care for our environment is part of BPRC's policies. We have an environmental management system which is ISO14001 certified (ISO14001:2015) and through which we manage and continuously try to reduce our environmental footprint.

Key aspects of BPRC's Health, Safety and Environmental policy are:

- Microbiological safety; preventing employees from being infected
- Protecting the environment from the release of biological agents (including GMOs)
- Restricting the use of hazardous substances
- Efficient energy and water consumption.

We consider the following aspects integral components of our operations:

- Continually raising awareness of safety and environmental concerns and providing information on these concerns to all employees and guests
- Appointing employees tasked with carrying out the duties arising from our Occupational Health and Safety and Environmental Protection Policies
- Informing employees and others of the quality of our work
- Creating the right facilities and ensuring that installations and equipment are used properly
- Identifying, recording and properly addressing complaints regarding health-and-safety and environmental protection issues
- Having annual internal audits performed.

Health, Safety & Environment

Energy consumption

In recent years BPRC invested in the reduction of energy consumption, for example by replacing light sources for more efficient LED-lights, an effort that has continued in 2023.

Safety awareness & training

Safety awareness is an essential part of the daily routine and BPRC staff is periodically trained in all aspects of safety.
For example; a training for radiological safety has been organized for all personnel that work with, or in the vicinity of, ionizing radiation which is part of the experiments involving our PET-CT.

BPRC's Research Areas

Modeling potentially life-threatening human diseases in non-human primates requires extended knowledge and dedication in animal care taking, colony management and translational research. Scientists from BPRC are world-wide acknowledged for their expertise in the translation of human diseases caused by viruses and parasites to non-human primates, as well as autoimmune diseases and genetics.

Research Areas
Alternatives

Monkeys are similar to humans. Not only on the outside but also on the inside.
That is because monkeys are genetically related to us. Due to this evolutionary relationship monkeys sometimes are a good model to study human diseases. But only if there is no other way.

Working with monkeys brings a great responsibility. We are responsible for the well-being of the animals in our colonies. We continuously seek to conduct research that does not involve animal testing in order to reduce the numbers of animals we work with. In the meantime, we accommodate and look after our monkeys with the best possible care.

We do this using the principles of the 3Rs. Refinement, reduction and replacement. Refinement and reduction go hand in hand as Refinement of an animal model will lead to a Reduction of the number of animals per experimental group.

3Rs throughout BPRC

Refinement

- Improvement of animal welfare is a continuous process in our institute. BPRC staff take part in (inter)national training programs to remain their high standards and gain new insights.
- All animals are socially housed.
- Stress is not good. It affects animals in breeding groups and can even affect the results of an experiment. In order to avoid stress you need to identify stressful events. And for that you need unbiased, objective and reliable parameters to determine stress.
    - Measuring the cortisol levels in hair samples is a method that can provide stress information from an individual animal. By cutting a hair into smaller pieces you can relate the cortisol levels to potential stressful events.

Research Areas
Alternatives

  - We take pictures as an objective measure for alopecia. Alopecia (hairless body parts) can be a sign for acute stress. Caretakers are trained to detect this and to take pictures. Sometimes an animal experiences stress from hierarchy in their breeding group. If that is the case behavioral scientists are notified to monitor the breeding group and if possible take measures.
  - When animals are prepared for housing in an experimental setting they are introduced to a selected cagemate. We can use round the clock camera recordings to monitor their behavior in the absence of a caretaker. This avoids less-compatible pair-housed animals.
- Positive reinforcement training (PRT). We have trained 25 animal caretakers how to train their animals. They do this twice per week. With this training method we are able to perform certain biotechnical techniques without sedating the animal.
- All marmosets jump voluntarily on a scale. This way their body weight can be monitored without sedation.
- All experimentally housed animals were trained to drink from a syringe, thus voluntarily take oral medication.
- Caretakers spent 15% of their time on (cage)-enrichment. For instance assembling food-puzzels, providing animals with toys or redecorate enclosures.
- Further improvements were implemented in diet variation, to maximize natural feeding routines.
- In 2017 an improved version of the ‘Welzijnsevaluaties’ was implemented.
- New features were introduced in our monkey database for the daily registration of each individual animal.
- All animals in experiments are observed at least twice a day. During this observation different parameters are ‘scored’. Normally an animal shows a broad variety of natural behaviors. In some models for (infectious) diseases the animal’s behavior changes. This is however a subjective parameter and changes are difficult to observe. Subtle changes during an experiment can provide crucial information. In this case we prefer to measure physical activity with telemetry. These devices register X-Y-Z coordinates of individual animals. If necessary it is also possible to measure body temperature, heartrate, blood pressure. This will lead to further refinement of our animal models.

Research Areas
Alternatives

Reduction

Optimizing and standardizing in vitro laboratory tests play an important role in the reduction of the animals we work with. Also in 2018 we have implemented new techniques. By using these new conditions, we aim at less variation in laboratory tests that will lead to smaller group sizes in our animal experiments.

Genetics

Genes play an important role in infections and diseases. We have implemented new techniques to determine the genetic background of animals in the breeding and experimental colonies. This enables us to select (or deselect) appropriate animals to answer particular research questions. For example; we know that certain genes play a role in the development of AIDS after HIV infection. We now know that these genes are also present in monkeys. Selection of animals for an HIV experiment is therefore based on these genes. Proper selection reduces the variation in an experiment and therefore smaller group sizes are required to obtain statistical significant differences.

Statistics at BPRC

One of the hallmarks of good science is statistics. Not only at the end of a proof on concept study to determine whether an HIV-vaccine was successful but also during the design of the study. Therefore, good statistics is part of the 3Rs.

Statistics is often used to determine whether differences in study outcomes are (statistically) significant. This is normally done by rejecting or accepting the null hypothesis, where the null hypothesis states that treatment does not have a significant effect. To do so, the p-value is calculated. If the p-value is below 0.05, the chance that the study outcome arose by chance is smaller than 1 in 20. In that case, the null hypothesis is rejected, supporting the alternative hypothesis that the observed difference was due to the treatment.

But statistical testing is only informative if the study is properly designed. If group sizes are too small a real difference may not be detected and the study will not be informative. If group sizes are large differences will be detected, but at the cost of too many animals. Therefore study design involves, amongst other things, also a so called “power calculation”.

Research Areas
Alternatives

The number of animals per group is calculated based on the desired effect of the treatment on the primary outcome (e.g. diseased or not-diseased), the between-animal variation of the treatment effect and the desired power. The desired power is the chance that a real difference, if present, is detected. This is usually set at 80% (i.e. 80 out of 100 studies will yield significant results). Next to the power calculation, the study design also involves methodological topics like randomization of the animals (treatments are allocated by chance) and blinding of observers (treatment is not known). Next to the power analysis, a statistical analysis plan is written before the study is performed. Because monkey studies are often the last step before testing in humans, monkey studies should be designed, performed, analyzed and reported in a similar fashion as clinical trials in humans.

PET-CT
ositron emission tomography–computed tomography (PET-CT) is a visualization technique that combines anatomic localization (X-ray) and functional imaging (nuclear medicine). In hospitals, PET-CT is already widely used during the diagnosis and treatment of cancer. Over the last years, PET-CT also proved its additional value to biomedical research with animals.

PET-CT offers many advantages over traditional techniques. First, PET-CT is minimal-invasive. Second, as results from blood tests, biopsies/swabs or cells washed out of the organ of interest can be indicative for infection, they are often poor indicators for actual disease manifestations. Besides biopsies only provide information of the tissue in the biopsy but often not of the entire organ. The combination of X-ray and specific radioactive probes allows screening of the entire body in both an anatomical and functional way. This minimizes the discomfort of the animals and provides you a much broader view.

In addition, PET-CT offers the opportunity to visualize disease progression or therapeutic response over time (longitudinal). This is particularly relevant when critical organs need to be studied, like lungs or brains. PET-CT in combination with 18F-Fluorodeoxyglucose (FDG) as imaging agent is well-established used for about 90% of the PET-CTs obtained in human. FDG visualizes the glucose metabolism in the body and shows increased signal in areas with

Research Areas
Alternatives

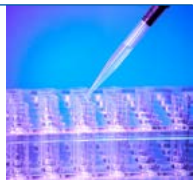
an increased metabolic activity. Increased metabolic activity can be due to cancer, infection/ inflammation though also after a surgery in the area where a scar is healing. This makes FDG PET-CT highly sensitive for detecting for instance tuberculosis and influenza in the lungs.

BPRC already started to use PET-CT in 2017. Initially only in our tuberculosis research but currently we are applying this state-of-the-art technique also in other research programs like influenza. With this, PET-CT is not only leading to new and more extensive scientific insights though also increasing the translational value of our animal models as PET-CT is a well-accepted imaging method in humans.

Replacement

In 2009 BPRC-researchers developed an new in vitro assay to test drugs for it's anti-malaria activity. This assay replaces the use of monkeys. Last year we tested 33 new potential anti-malaria drugs with this assay. Before 2009, 33 monkeys would have been necessary to test these 34 compounds. So far, BPRC tested 999 drugs with the animal-free assay.

Research Areas
Alternatives



3Rs Alternatives Unit BPRC

At BPRC, we are fully aware of our responsibility to animals and society. We use animals for our research only to study essential questions around serious and life-threatening diseases for humans and when these questions cannot be answered with non-animal methods or with animals other than monkeys.

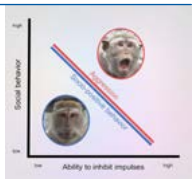
Alternative methods are categorized along the principle of the 3Rs of Replacement Reduction and Refinement, all of which have a place within BPRC. BPRC is committed to the implementation of the 3Rs and is actively involved in the development and implementation of 3Rs methods. The 3Rs are implemented in the research of every department.

BPRC is currently restructuring the Alternatives department to be able to respond even better to developments in the field of alternatives and implementation of the latest techniques. Recently, a new Head of Department has been appointed. She will be the connecting person for the different activities of the various departments regarding alternatives and be actively involved in the development and implementation of new methods in this rapidly developing research field.

Research Areas
Ethology

Monkeys are social animals that both compete and cooperate with their group members. Understanding the dynamics in behaviour of monkeys is not only important for scientific behaviour research, but also to manage our breeding and experimental colonies.
To improve our knowledge, we work together with a group of behavioural scientists from the University of Utrecht.

The ability to inhibit impulses is related to social behaviour in long-tailed macaques



Performance in cognitive tasks has been linked to differences in species' social organization, yet to understand its function its relationship to within-species variation in behavior should also be explored. One important cognitive capacity, the ability to inhibit impulses, is typically better in egalitarian than despotic primate species and in primate species with strong fission-fusion dynamics. A different line of research indicates that a high ability to inhibit impulses is related to less aggressive behavior and more socio-positive behavior. However, within species the relationship between performance on cognitive inhibition tasks and variation in social behavior remains to be explored. Here we investigate how performance in a typical inhibition task in cognitive research is related to aggressive and socio-positive behavior in despotic long-tailed macaques. Twenty individuals living in two naturalistic mixed- sex groups were tested with the Plexiglass Hole Task. Aggressive behavior and three types of socio-positive behavior (neutral/friendly approaches, socio-positive signaling, and grooming others) among group members were measured. Individuals differed in their ability to inhibit impulses. Individuals that were not good at inhibiting impulses showed higher rates of aggressive behavior, but also more socio- positive signals, whereas inhibition was not related to neutral/friendly approaches and grooming. These results confirm the positive link between impulsiveness and aggression. In addition, the results indicate that some social-positive behavior may be enhanced when inhibition is limited.

Research Areas

Ethology

In this species, benefits potentially derived from aggression and socio-positive signals match a low ability to inhibit impulses, suggesting that a low ability to inhibit impulses may actually be advantageous. To understand differences between species in cognitive skills, understanding the benefits of variation in a cognitive capacity within a species is crucial.

Read more >

Research Areas

General Primate Biology & Welfare

Maintaining the health and stability of the monkeys in our self-sustainable breeding colonies and research facility requires dedication and expertise. This is a joint effort between animal caretakers, veterinarian staff, ethologists, laboratory staff and experts in genetics.

Colony Management

Over the last few years, the macaque breeding colonies have decreased in number of individuals due to the high demand on experimental animals (i.e. for SARS-CoV-2 research). In 2023 our goal was not only to enlarge the breeding colonies, but also to have the genetic variability large enough to keep them self-sustaining over the next decade.

To increase the number of rhesus macaque offspring, 4 genetically characterized adult males were introduced into breeding groups. At the end of the year, the colony had 17 out of 24 rhesus breeding groups with an active breeding male. A total number of 112 rhesus babies were born. In the long-tailed macaque breeding colony, 28 babies were born in 6 active breeding groups.
In September 2023, at the start of the next breeding season, preparations were made for two additional male introductions.

For an adult macaque male, introduction into a new breeding group is very costly. Sometimes females react quite aggressively toward the unfamiliar male, and during the introduction period (which can take up to 2-3 months) the decrease in bodyweight of the male is significant. These introductions are therefore intensively supervised by colony management; a specialized animal caretaker is present during the day to intervene when necessary. With camera's the behaviour of the animals is recorded, even throughout the night. To prevent the male from losing to much bodyweight, the animal trainers have trained the male to accept so-called "brokkenballen" with extra high caloric food.

Research Areas

General Primate Biology & Welfare

His bodyweight is monitored by voluntary weighing. The extensive expertise in observational and animal training techniques as well as substantial time investment by the ASD staff is essential to ensure animal welfare.

Veterinary care

In collaboration with the faculties of Veterinary Sciences at Ghent University and Utrecht University, we completed the second phase of the long-acting antibiotic pharmacokinetics study of longacting ampicilline. Preliminary results indicate that longacting ampicilline achieves sufficient serum levels in rhesus macaques, suggesting that its long-acting properties can reduce treatment frequency, thereby improving welfare in group-housed rhesus macaques.

Our implanon research project, completed and published in 2023, included a comprehensive analysis of blood chemistry, effectiveness, and the impact on uterine volume and endometrial thickness measured by ultrasound. Additionally, the University of Nebraska Medical Center analyzed synthetic progestin concentrations in both rhesus macaques and cynomolgus macaques carrying implanon. This project resulted in the first peer-reviewed publication that scientifically demonstrated the effectiveness of this contraceptive method for optimal birth control in macaque breeding colonies.

From 2020-2022, we conducted a randomized diet study in rhesus macaques with idiopathic diarrhea. Fecal samples were whole-genome sequenced in collaboration with TNO, with further analysis by HORAIZON technology. State-of-the-art machine learning techniques were applied to research possible microbiome signatures of animals with idiopathic diarrhea. In 2023 we further analyzed these data that we will finalize in 2024. The involvement of various nutrients and potential effect on diarrhea is currently studies

Research Areas

General Primate Biology & Welfare

in collaboration with the faculty of veterinary sciences in Utrecht. Together, our data will contribute to finding solutions to reduce the occurrence of idiopathic diarrhea in macaques housed in animal centres and zoos.

We used our historical database to determine reference values for hematological and biochemical parameters in different age groups and genders of macaques. This allows us to determine the health status of the animals in a very precise way.

Read more >

AI

Recently, BPRC in close collaboration with the group Animal Behaviour and Cognition (Biology, Utrecht University) has started various projects using artificial intelligence to start automatic recognition of animals and their behaviour. Although it's still in the early stages, we hope that next to behavioural research in due time we can also use these AI methods to enable us to assess welfare of the animals in an unbiased way.

Research Areas

Medical imaging

The aim of experimental animal models is to understand infection and disease to enable effective prevention or treatment. Our studies in NHPs include research on various physiological and anatomical aspects of diseases, such as infectious diseases, neurological and aging-related diseases, including pathogen dynamics, immune responses, and host pathology. To study these various aspects, non-invasive imaging techniques, such as Chest X-ray (CXR), computed tomography (CT), and positron emission tomography - computed tomography (PET-CT) are applied. These imaging possibilities allow longitudinal follow up, thereby contributing to reduction of the number of animals.

In 2023 immunoPET was introduced in the BPRC by imaging inflammatory processes with the use of five different PET tracers. These were tested in two SARS-CoV-2 infection studies and in one tuberculosis study (all three studies are in preparation to be published). In the SARS-CoV-2 studies four complete newly developed tracers were tested to longitudinally visualize the infection process not only in the lower respiratory system but also the rest of the body. All tracers were safe to use and were suitable to detect alterations in the body after a SARS-CoV-2 infection. Anti-Spike nanobodies proved useful in monitoring potential sites of virus replication in the nasal mucosa and lung, while the accumulation of MHC class II and CD8 nanobodies in non-affected lung tissue and other lymphoid and non-lymphoid tissues may form an indication of triggering of innate and adaptive immune responses.
In addition, with the use of two different macrophage/monocyte directed tracers the different responses of the body towards this subpopulation of immune cells were visualized.

Research Areas

Tuberculosis

Tuberculosis (TB) is a bacterial infection that causes lung disease. TB is still one of the deadliest infectious disease by a single pathogen in the world. Progress made in the years up to 2019 has slowed, stalled, or reversed, and global TB targets are off track (WHO report 2023). The global estimates of the number of deaths caused by TB has now gone down to the level of 2019 and has decreased toFor the first time in over a decade TB death increased in 2022 to 1.3 million in 2022. Fewer people were diagnosed and treated or provided with TB preventive treatment and fewer resources (*funding*) for essential TB services and R&D were available. In addition, we are still confronted with the fact that approximately 25% of the world population is latently infected with TB.

Antibiotics are currently the only treatment options in bringing the disease down. However, anti-microbial drug resistance in TB is on the rise, which makes it harder to treat. Overall, a growing number of TB infected people cannot be treated and die of the disease. Prevention by vaccination is important to break the cycle of TB transmission and infection.

Read more >

BPRC continues to use nonhuman primate models of TB, specifically the rhesus monkey and the cynomolgus monkey, to get a better insight in how the disease develops and to evaluate new treatments of TB. TB in these 2 species represents two different disease manifestations. TB in rhesus monkeys develops as a progressive active form of the disease, while infection of cynomolgus monkeys can also develop as a latent disease.
Together, these models best recapitulate the range of manifestations of tuberculosis in man.

Research Areas
Tuberculosis

Local administration of BCG-vaccine is superior to classical skin immunization continued

Vaccines aim at the induction of long-lived immune memory specific for the disease you vaccinate against. This memory is mediated by the adaptive immune system consisting of T and B cells. The role of the innate immune system in the context of vaccines is primarily considered to provide the proper help to stimulate the adaptive response. Innate immune responses and function were considered constant and invariable over time. Over the last decade, however, it has become clear that also innate immune cells, like monocytes, macrophages and NK cells can adjust and display memory-like phenotypes. This innate immune 'memory' is now typically referred to as trained immunity. BCG is the prototypical vaccine that, next to a TB-specific effect, can induce trained immunity and provides broad (heterologous) protection against disease from other respiratory infections.

We have previously shown that mucosal vaccination with BCG delivered in the lung provided better protection against TB than classical immunization in the skin and that this protection was associated with unique TB specific local adaptive immune responses.

Read more > article and commentary.

We extended our analyses of the immune response observed after mucosal vaccination with BCG and established that these vaccine-induced T cells in the lung were endowed with increased expression of homing markers and tissue residency markers.

Read more >

Research Areas
Tuberculosis

We showed for the first time in nonhuman primates that we can recapitulate the trained immunity phenotype observed in humans after standard TB vaccination in the skin.
In addition, we demonstrated that mucosal vaccination through the lung results in improved trained immunity compared to the standard vaccination.

Read more >

The new vaccine candidate MTBVAC that was derived form a human clinical isolate of mycobacterium and is now in phase III of clinical development, was equally potent in the induction of trained immunity after mucosal vaccination as BCG.

Read more >

To make mucosal delivery in the lung translatable to the clinic we further demonstrated that aerosol delivery, of MTBVAC with a nebulizer, instead of endobronchial instillation, provided robust protection against TB disease compared to non-vaccinated control animals. MTBVAC delivered through aerosol induced comparable immune signatures to what we have seen before upon pulmonary mucosal MTBVAC instillation. (manuscript in preparation).

These results provide support to strategies for improving TB vaccination and prompted us to further investigate whether the mucosal whole cell vaccination could be used for revaccination purposes.

Standard intradermal BCG vaccination is given to millions of children every year to protect them from TB in their childhood. However, at the age of adolescence, the protective efficacy of revaccination standard intradermal BCG against pulmonary TB is highly variable ranging from 0-80%. Altering the route of vaccination in adults (revaccination) might be a way to improve efficacy in these adults.

Research Areas
Tuberculosis

We established that mucosal BCG revaccination after a standard intradermal BCG vaccination resulted in a reduction of TB disease and that this was associated with the same unique local immune signature as was observed with mucosal vaccination in naïve animals (manuscript in preparation).

In order to study revaccination in adults that were exposed to BCG at birth, which is a relevant clinical situation, we have created a cohort of rhesus monkeys vaccinated at birth. This project started in 2020 and after 4 years the first cohort of neonatal vaccinated animals is becoming available to test revaccination strategies by alternative routes. Neonates had a positive immune response to the BCG when tested ± 10 weeks after standard intradermal BCG vaccination. We also found elevated innate responses in these young animals when tested ± 10 weeks after BCG vaccination. Both adaptive and innate immune responses waned over time and reverted to baseline levels comparable to what is seen in non-vaccinated cohort controls.
Our group, as partner in a TBVI governed network has been awarded funding to perform a study to compare mucosal respiratory vaccination strategies in human, rhesus monkeys (nonhuman primates) and mice allowing for an extensive analysis of alternative route of administration increasing the vaccine efficacy.

In collaboration with expert US partners, we started a NIH funded project, in which we explored the use of nucleic acid DNA vaccine against TB. After the success of RNA vaccines in the fight against COVID we anticipated that the use of nucleic acid-based vaccines could also be used in the fight against TB. After primary BCG vaccination the animals received a booster vaccination with the pDNA.TB vaccine that contained 9 proteins derived from Mycobacterium tuberculosis (Mtb). The pDNA.TB was injected the skin followed by the application a small local electric current (electroporation) facilitating the uptake of the DNA by cells in the skin which then started the production of those protein. We established that the production of those protein resulted in a vaccine specific immune response. In the follow up the animals will be challenged with Mtb to establish whether this strategy is able to protect the animals against TB.

Research Areas

Comparative Genetics & Refinement

The immune system is a complex network of cells and tissues that work together to defend the body against pathogens. Certain types of molecules that are involved in the immune system display significant variation, resulting in diverse immune responses in individuals and populations. This contributes to differences in susceptibility or resistance to pathogens and disease. The observed variation in these types of molecules is encoded in genes and are called “polymorphisms”. These polymorphisms ensure unique immune responses, thereby preventing the elimination of an entire population by a single pathogen.

Key components of the immune system that display extensive polymorphism, such as the killer cell immunoglobulin-like receptor (KIR), the leucocyte immunoglobulin-like receptor (LILR) families and the major histocompatibility complex (MHC). An effective immune response relies on the cooperation between different components, like MHC and KIR interactions. In general, MHC molecules are involved in the presentation of fragments of proteins (peptides) from one’s own tissue (“self”) and/or from pathogens (“non-self”) to immune cells. This process facilitates the recognition of pathogens and triggers an effective immune response. The KIR and LILR molecules finetune immune responses and serve as monitoring mechanism (immune checkpoint) for pathogen evasion. Two other important components of the immune system, displaying substantial variation are the T- and B-cell receptors (TCR and BCR). Also, this variation is crucial for the recognition and targeting of specific pathogens. The broad diversity recorded for different components of the immune system determines health and disease at the level of an individual.

Monkeys share a high genetic similarity with humans, especially for the immune system components. Studying genetic polymorphisms in monkeys and their impact on the immune system provides valuable insights into the functionality of immune defence mechanisms in humans. This knowledge is particularly important in developing personalized medicines tailored to individual genetic profiles.

Research Areas

Comparative Genetics & Refinement

Within the Comparative Genetics and Refinement department, our research focusses on the characterization of complex immune regions, such as the MHC, KIR, and LILR genes, in different monkey and great ape species. Also, we started to investigate the BCR and TCR diversity at a genomic and transcriptomic level. We use the latest DNA sequencing strategies, utilizing different PacBio and Oxford Nanopore Technologies platforms, in combination with other molecular techniques, like fragment analyses on short tandem repeats (STR). This will allow us to study repertoire expansion and contraction during vaccination and infection studies in experimental models for disease.
In addition to studying the genome and transcriptome from animals in our breeding colonies, we also examine on request samples from other institutions and zoos.

The newest molecular techniques to unravel the most complex regions of the genome

The sequencing techniques have rapidly evolved in recent years, which allows us to characterize the most complex regions in the genome at high resolution. Conventional short-read sequencing strategies have shown their value in the high-throughput typing of the MHC and KIR gene systems but was insufficient to uncover the entire complexity. With the implementation of long-read sequencing platforms, developed by Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT), the plasticity of the immune regions could be unravelled. At the CG&R department, we have introduced these platforms to study the MHC, KIR and LILR families at genomic and transcriptomic level.

In a review, we have described how these techniques have revolutionized the characterization of MHC and KIR in humans and non-human primates.

Read more >

Research Areas

Comparative Genetics & Refinement

In 2021 we have applied one of these long-read sequencing approaches in combination with a Cas9-mediated enrichment protocol. Using specific guiding RNAs in complex with a Cas9 nuclease enzyme, double-stranded breaks could be introduced at the target region to subsequently initialize the ligation of sequencing adaptors. This protocol enriched for large overlapping DNA fragments without amplification. We succeeded in the assembly and phasing of entire human and rhesus macaque KIR haplotypes. This enrichment protocol is adaptable to any species, as we demonstrated by its recent application in chimpanzees.

From an evolutionary point of view, chimpanzees are an interesting species to study. Previously, we showed that the chimpanzee MHC class I repertoire has been skewed due to an ancient selective sweep. In this same group of animals, we set out to determine the KIR repertoire, to deepen our understanding on the sweep's extent. In this study, we utilized a PacBio platform to sequence the full-length KIR transcriptome and uncovered phased KIR haplotypes by applying the afore mentioned enrichment approach in concert with long-read ONT sequencing. Overall, the transcriptomic and genomic analyses demonstrated a reduced variability of the KIR repertoire in chimpanzees in comparison to what has been published for humans and rhesus macaques. This suggest that the ancient selective sweep acting on the MHC class I repertoire may also have had its impact on the KIR system.
In addition, we have analysed the chimpanzee KIR repertoire from a functional perspective. Chimpanzees mainly exhibit KIRs with inhibitory potential. Our population data showed a skewing of the inhibitory KIR repertoire towards specificity for C2, an epitope encoded on particular MHC class I molecules. This observation corresponds to the higher percentage of C2-epitope-bearing MHC class I molecules identified in chimpanzees, contrasting with a general predominance of C1-epitope-bearing MHC molecules in humans. However, an exception is formed by African populations, with a higher frequency of MHC class I molecules comprising the C2-epitope. Also, in bonobos, another species native to the African continent, a skewing towards a C2-mediated immune response has been observed. These findings indicate that similar selective forces are at play across these three specific populations, which are most likely driven by endemic pathogens such as HIV-1/SIV and the malaria parasite "*plasmodium falciparum*".

Read more >

Research Areas

Comparative Genetics & Refinement

Understanding the genetics of the B-cell receptor to refine vaccine-development studies

Painted by S. Ott

Antibodies play a crucial role in the defence against extracellular stage of infections caused by pathogens. Therefore, many vaccination strategies aim at the induction of (neutralizing) antibody production. In primates, functional antibodies are dimers composed of a light and a heavy chain. The genes that encode the different chains are located on separate chromosomes, and antibody formation is controlled by a complex genetic process that is called gene rearrangement. In the case of the heavy chain, the relevant gene segments have been designated V (variable), D (diversity) and J (joining) and C (constant), whereas the organization of the light chain is similar but lacks the D segment. A commonly neglected aspect is the substantial variation in the number of V genes among individuals. Recent studies indicate that during an infection or autoimmune disease preferential V segment-usage may occur. The process itself is, however, poorly understood.

As part of a PhD project, and in collaboration with the Virology department of the BPRC, we started to characterize the genomic region encoding the BCR segments in rhesus macaques. Using a hybrid sequencing approach, combining the strengths of PacBio and ONT platforms, the BCR regions located on different chromosomes are assembled and annotated. At present, we have successfully designed a characterization protocol that has been applied to a rhesus and a cynomolgus macaque, and more individuals will follow to uncover the diversity at the BCR and TCR regions in macaque species housed at BPRC. Eventually, this genomic data will be examined in concert with BCR transcriptome data from infection and vaccination studies to monitor the immune response in detail. This knowledge will advance the interpretation of study outcomes, thereby facilitating a better translation of these preclinical studies into practical applications.

Research Areas

Comparative Genetics & Refinement

Bioinformatics: streamlined pipelines for improved data analysis

The implementation of long-read sequencing results in an increasing amount of data that has to be analysed and stored. The CG&R department is committed to data analysis, visualisation, and storage through the development of tailored data-analysing pipelines. This involves a broad range of projects, including the automated assembly and annotation of phased immune regions, such as KIR, BCR, and TCR haplotypes.

Streamlined pipelines also provide opportunities to delve deeper into data, providing more detailed information from experiments. By employing sophisticated computational methods, patterns, correlations, and information could be uncovered that might otherwise go unnoticed. In addition, a more detailed analysis of the different immune regions might also enhance the studies of other departments within BPRC, aiding in the interpretation of their outcomes. For instance, a comprehensive characterization of specific immune regions, or even entire genomes, may improve the annotation of single-cell RNA-seq or ChIP-seq sequencing data. As such, the development of streamlined pipelines maximizes the value of each experiment and thereby contributes to the refinement of studies at BPRC.

Research Areas

Influenza

Influenza (flu) is a contagious respiratory disease caused by influenza viruses. It can cause mild to severe illness. Every year over half a million people die of seasonal influenza. Many different influenza viruses are found around the globe and these viruses easily mutate to new virus variants, the so-called ‘antigenic drift’.

In addition, there is the constant threat of a new pandemic influenza virus. A ‘new’ virus that may be formed after recombination between bird-influenza viruses and pig-influenza viruses, and that is able cause serious disease in humans. A scenario similar to the Spanish flu in 1918 which killed over 50 million people. This is called ‘antigenic shift’.

Ideally, an influenza vaccine provides protection against a broad spectrum of seasonal influenza, as well as pandemic influenza viruses. However, current influenza vaccines afford only limited protection against seasonal as well as pandemic influenza. Therefore, new and improved vaccine-strategies are required. This involves new vaccine concepts and improved vaccine production technologies.

At BPRC we use influenza infection models in monkeys to evaluate the protective capacity of novel vaccine strategies.

Research Areas
Influenza

Experimental animal models for universal influenza vaccines

Non-human primate animal models are important to determine whether novel vaccines against influenza can provide a good and broad protection against influenza virus and are safe to use. To measure protection, it is necessary to experimentally expose animals to influenza virus. This is usually done by applying an amount of virus in the nose, mouth and directly in the lungs. However, infection in humans is mainly caused by exposure to aerosols or droplets that enter the airways either via respiration, inhalation, or via contact with contaminated surfaces. To better mimic this typical human way of exposure, a non-human primate model was developed that could be infected by virus in aerosols. Although the animals became infected, the reaction of the body differed from what was observed when the virus was directly injected into the lungs. Infection by aerosols gave lower levels of inflammation and may therefore be more typical of a mild infection in humans. Instead, direct injection in the lungs gave more pronounced inflammatory response, typical of the more severe infection that develops in some humans. These findings can contribute to using the correct animal model for testing of vaccines for use in humans and better prediction of the effect that a vaccine will have in humans. This work is published in Journal of General Virology.

Read more >

Research Areas
Influenza

Experimental animal models for bird flu

The name bird flu, or avian influenza, is misleading as bird flu virus not only infects birds but occasionally also humans. Bird flu virus infection of humans is rare,
but if it happens often fatal i.e 455 deaths in a total of 861 cases for so called H5 viruses.
Like for seasonal flu, it is crucial to have an animal model to test vaccines.

In 2020 we used various techniques to expose macaques to H5N1 bird flu virus.
The results of that work are now prepared for publishing.

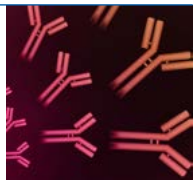
Vaccines and antiviral drugs

In 2022 we started a study to investigate a new vaccination regimen against Flu and RSV (Respiratory Syncytial Virus). RSV is the commonest cause of lower respiratory tract infection in children. Worldwide an estimated 34 million children fall ill due to RSV each year and over 3 million children have to be taken to a hospital and 66,000–199,000 children die. We tested whether vaccination via a spray on the tonsils could induce more long-lasting protection against infection with RSV as well as influenza. In addition, we tested whether this vaccine strategy could provide broader protection against influenza, which is needed because of the continuous formation of new virus variants (see above). After an initial intramuscular injection with vaccine, two boosters were administered to the respiratory tract as a spray. Animals were then experimentally infected with RSV. The vaccine strategy proved to result in lower levels of virus replication in the nose, throat, and lungs. In 2023 it was tested whether this vaccine strategy could also protect against influenza. However, no difference in influenza virus replication was observed. The difference between protection against RSV and no protection against influenza may have been due to the different roles

Research Areas
Influenza

of cellular immunity versus antibody mediated protection. Both against RSV and influenza we could measure strong cellular responses in the lungs, but no antibodies. In 2023 an experiment was started to test how effective this new vaccine regimen is against SARS-CoV-2.

PhD student influenza-specific antibody responses



Protection from influenza infection relies on good antibody responses.
As part of a PhD project and in collaboration with the Amsterdam Medical Center novel influenza proteins were designed by a BPRC PhD student, to identify precisely which antibody-producing B-cells are induced by vaccination or influenza virus infection. This work describes the method to characterize the diversity of antibody responses against influenza. It will be applied on frozen materials from previously performed animal studies to identify protein specific B cells and antibodies with unknown specificities that could be relevant for vaccine design.

The novel influenza proteins were used to study how B cell responses against influenza are formed during a first infection. This aspect is difficult to study in humans, because the first infection always occurs early in life and adults have already encountered several different viruses. However, it is important to understand how a first response is formed as the first response is thought to determine how one responds to subsequent viral infections. Frozen materials from previously performed influenza infection studies in macaques were used to study how the first response is formed.

It is known that after influenza infection most of the antibodies that are formed are directed against the hemagglutinin molecule. Hemagglutinin is present on the outside of the virus particle and is necessary for the virus to bind to its target cells and to infect these cells. Hemagglutinin is composed of a highly variable head domain and a much more constant

Research Areas
Influenza

stem domain. The head domain is needed for the binding to the target cell. It is at this site that most of the mutations occur, leading to new virus variants that are no longer recognized by the antibodies that were formed during a previous infection or vaccination. This is why people need to be vaccinated with a new vaccine every year. The stem domain is much more constant and is needed for virus entry into the target cell. Antibodies directed against this part of the hemagglutinin protein are able to recognize different virus variants, but they are less efficient in blocking the virus than the anti-head antibodies. How to induce these types of antibodies via vaccination is one of the key-questions in influenza vaccine development. We have shown that such antibodies are formed after the first infection with influenza virus. However, we found that they strongly decrease in time, while the anti-head antibodies gradually increase and come to dominate the antibody response. These results were recently published.

Read more >

In 2023 we tested whether immunization with hemagglutinin from five different influenza strains could induce cross-protective responses against a very different influenza virus. If successful, then this could help to design better influenza vaccines for humans that would give broad protection and thereby make yearly vaccinations no longer necessary. Unfortunately, there was no protection against infection. No anti-stem antibodies could be detected after immunization. However, after the infection there was a strong increase in anti-stem antibodies and they were higher in the immunized animals than in the infection controls. Therefore, the immunization did have a priming effect resulting in higher responses after breakthrough infection.

Research Areas
Influenza

It is thought that our immune response is strongly affected by the particular virus strain that is causing the first infection in life. This is called “original antigenic sin” and could hamper the efficacy of vaccination later in life against newly arising different virus strains. However, we observed that there is a limit to “original antigenic sin” and that it does no longer play a role when the genetic difference between first and second virus strain is too large.
These results were recently published.

Read more >

Further research will be directed at understanding how stable these responses against influenza are in time. This cannot be studied in humans as they are continuously exposed to new influenza viruses. In addition, the precise function of the influenza specific antibodies will be further investigated. In 2023 a new PhD student has started to work at the BPRC to continue these studies.

Research Areas

Rabies Virus

Rabies is a devastating viral disease caused by the rabies lyssavirus (RABV). RABV is transmitted to humans via a bite by infected animals, predominantly dogs, but foxes, raccoons, and bat species also serve as natural reservoirs. In humans, once the first clinical symptoms have developed, the disease is uniformly lethal, and patients die in great agony. Rabies causes 58.000 human deaths every year, mostly in rural areas of Africa and Asia. The majority are children of young age (<15 years), who are at high risk from being bitten while playing with animals.

In the last quarter of 2021, we evaluated the immunogenicity of two rabies virus vaccines which were built on the backbones of the attenuated yellow fever virus vaccine strain, YFV-17D, and the Japanese encephalitis virus vaccine strain (CD-JEVAX). The first analyses show that both vaccines effectively induced humoral immunity, equal or exceeding those induced by commercially available rabies vaccines. Further analyses are ongoing, and results will become available in 2022.

In 2022, the analysis from many samples obtained from the animal study performed in 2021 has been finalized. A manuscript is being prepared for publication.

Research Areas

SARS-CoV-2 and COVID-19

In 2020, the world was startled by SARS-CoV-2, the causative agent of COVID-19. The department of Virology set up a research line to investigate the pathogenesis of this new infection and collaborate in the development of new vaccines to combat the pandemic.

No animal-free alternative

Vaccines stimulate the immune system in such a way that when it encounters the real pathogen, our immune system responds fast and efficient to prevent infection or disease. The immune system is a complicated 'organ' that involves thousands of different cells and molecules. At this point in time, it is not possible to mimic the interplay between all these different components outside a live body. Therefore, efficacy of vaccine candidates is tested in an animal model.

Coronaviruses have limited host range

Coronaviruses, like SARS-CoV-2, are known for their limited host range, but also for their potential to cause serious disease in humans when spilled over from their natural hosts. SARS-COV-2 is not the first coronavirus to cause severe illness in humans. In 2003 there was an outbreak of SARS with a total of 8096 registered infections of which 774 people died. And in 2012 it was MERS with 2494 cases, including 858 fatalities. SARS and MERS are known to infect macaques, and experimental infection results in lung pathology resembling pneumonia in humans. So, when the dramatically fast spread of SARS-CoV-2 became evident, it was clear that a vaccine was urgently needed. With the knowledge that macaques are one of the few animal species susceptible to coronaviruses that cause disease in humans, macaques became one of the preferred animal species to investigate crucial steps in SARS-CoV-2 drug- and vaccine development.

Research Areas

SARS-CoV-2 and COVID-19

SARS-CoV-2 in macaques

In 2020 we established a nonhuman primate model for SARS-CoV-2 infection. One of the first papers showing that macaques were susceptible to SARS-CoV-2 was co-authored by BPRC and published in Science. This study particularly focused on the early events during CoV infection.

Read more >

We have extended this research by studying the post-acute phase of SARS-CoV-2 infection in two macaque species showing ongoing virus replication and pathology in lungs and other tissues even 5 to 6 weeks after infection which could be relevant for understanding the long-term consequences (like Long-COVID syndrome) of COVID-19 in humans.

Read more >

As an extension of this study, we investigated the neuropathological changes that occurred after a SARS-CoV-2 infection in the brains of macaques. Our data highlight the potential of the virus to cause pathology in the brain of macaques as in all animals we observed a range of neurological abnormalities, like hypermetabolic pituitary gland, α-synuclein inclusions, activated microglia in the brain parenchyma, and infiltrating T-cells. The heterogeneity of these manifestations in the brains shows the neuropathological potential of SARS-CoV-2 and should be considered a warning for long-term health risks, following SARS-CoV-2 infection.

Read more >

Research Areas

SARS-CoV-2 and COVID-19

Vaccines and antiviral drugs

In 2021, several SARS-CoV-2 vaccine candidates were evaluated in collaboration with academia and pharmaceutical partners. One of the first-generation vaccine candidates that was evaluated at BPRC has been approved by different national and international authorities for use in humans. This vaccine is now used worldwide as one of the first effective SARS-CoV-2 vaccines. The results of this efficacy study have been published in the Journal of Experimental Medicine.

Read more >

Four other SARS-CoV-2 vaccine candidates have been evaluated in 2021. Some vaccines were very effective in protecting against SARS-CoV-2 infection in our models. Several studies are published in 2022.

Read more >
Read more >

In 2022, we performed two COVID-19 vaccine studies using different new vaccines candidates under the sponsorship of the European TransVac-2. One study was done in collaboration with a consortium led by the University of Copenhagen (Denmark). The second study was in collaboration with a small biotech company from Belgium. Both efficacy studies were done in 2022. The results of one study were published.

Read more >

Research Areas
HIV-AIDS

AIDS is caused by an infection of the Human Immunodeficiency Virus (HIV). Antiviral medications can delay HIV growth and can prevent AIDS development. Furthermore, these compounds can prevent transmission from the virus from an infected person to a non-infected individual. However, we see that those antivirals are not able to prevent the spread of this pandemic worldwide as there are approximately 1.5 million newly infected people each year. Therefore, we hope that effective vaccines against HIV are more efficient in stopping the spread of HIV. Already more that 35 years have been passed to find an effective vaccine. Unfortunately, no effective vaccines have been developed yet, but the search continues.

The HIV prime-boost vaccination study against HIV, which started in 2020 as part of a large EU consortium, has been finalized this year. Despite the use of newly developed vaccine candidates (and the vaccination strategy tested), they failed to induce strong immune responses in the rhesus macaques. The candidates tested did not induce stronger and/or broader immune responses as compared to earlier evaluated vaccine candidates.
Still *in vitro* analysis is ongoing to investigate the (lack of) effectiveness of these latest vaccine candidates against HIV.

Research Areas

Mosquito-borne Diseases

Dengue virus, West Nile virus, Rift Valley fever virus, and Zika virus are mosquito-borne viruses that cause an infection in people. In most people, the infection is transient and without clinically relevant illness. However, approximately 1% of the patients suffer from complications. Nowadays, over 700 million people get infected with a mosquito-borne virus each year. Due to the growth of the human population and global warming this number is expected to increase dramatically over the next decades.

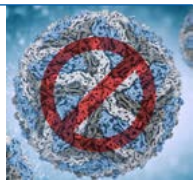
So far, vaccines are only available for dengue virus and yellow fever virus, but these vaccines have severe limitations and are not advised to people that run the highest risk for complications, namely children, older people and those people with an impaired immune system.

In time BPRC has developed several infection models to investigate mosquito-borne viruses. In 2020 these models were mainly used for proof of concept-studies for vaccines and antiviral drugs.

Research Areas

Mosquito-borne Diseases

An antiviral drug for dengue virus



There are four types of dengue viruses. Each of them inducing a somewhat different set of antibodies after infection. A first infection results in lifelong protection against this specific serotype. However, if the second infection occurs with another serotype the patient has an increased chance to develop severe disease. This phenomenon is called antibody-dependent enhancement (ADE). Severe dengue disease is a potentially deadly complication due to plasma leakage, fluid accumulation, respiratory distress, severe bleedings, or organ impairment. Every year, half a million patients require hospitalization and about 20,000 people die of severe dengue.

With a European partner we evaluated in 2021 their antiviral compound against dengue virus type 3 infection in our animal model. This compound had been tested in 2020 against one of the four dengue virus serotypes and proved very effective against serotype 2. This year we evaluated the same compound but now against serotype 3 dengue virus infection to determine its broad effectiveness.

In 2022, we continued together with our partner the evaluation of their antiviral compound, now against dengue virus type 4 to determine its broad effectiveness. The most important results from this collaboration have been published early 2023.

Read more >

Research Areas

Mosquito-borne Diseases

A new zika virus vaccine tested in rhesus macaques

The classic method of vaccine production is time consuming, requires batch-to-batch evaluation and distribution using a cold-chain. This hampers a rapid response in case of an outbreak. The 2015-2016 outbreak of Zika virus brought the weaknesses of classic vaccines to light.

A new generation of vaccines may overcome these problems. DNA vaccines are safe, can be rapidly produced and do not require a cold-chain. However, not all DNA vaccines are effective in inducing an immune response.

BPRC has collaborated in an EU-funded project to perform a proof-of-concept study in monkeys with a DNA vaccine encoding for the Zika envelope protein. Although the immune responses were high, the vaccine did not induce sterile immunity. A manuscript describing this study is currently in preparation.

Research Areas

Mosquito-borne Diseases

A new Rift Valley fever virus vaccine tested in common marmosets

Rift Valley fever virus (RVFV) is an emerging mosquito-borne virus that is highly pathogenic to wild and domesticated cattle and humans. While animals are exclusively infected via mosquito bites, humans can also be infected via contact with tissues or blood released during the slaughtering of RVFV-infected animals. No human vaccine is available and currently commercialized veterinary vaccines are not optimal for human use.
In collaboration with a Dutch partner, we tested new vaccine candidates for safety and their capacity to generate protective immune responses in common marmosets.
The marmoset was used because it is very susceptible to the virus, so that any safety risks can be adequately measured. In addition, testing in a non-human primate species makes it possible to better extrapolate the measured immune responses to the human situation.
The vaccines were found to be safe and to induce strong potentially protective responses.

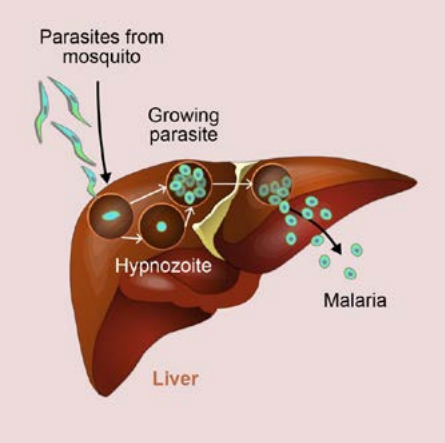
The analysis from data from this study was finalized in 2022, resulting in the publication of this project. A follow-up study is planned in 2024.

Read more >

Research Areas
Malaria

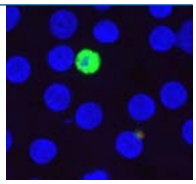
There are 5 malaria parasite (Plasmodium) species that infect humans, of which *P. falciparum* and *P. vivax* are the most important. *P. vivax* uniquely forms latent (dormant) parasite stages in the liver, called hypnozoites. Hypnozoites can become active through unknown mechanisms weeks to years after infection, resulting in malaria disease without being bitten by an infected mosquito (Figure). Hypnozoite infection itself is symptomless and cannot be diagnosed and thus forms a complication to malaria eradication.

Hypnozoites are only formed in 5 primate malarias (including 2 human malarias) and we use *P. cynomolgi*, that infects rhesus monkeys (and also humans as a zoonosis), as a model for *P. vivax*. We study hypnozoite biology and interactions with the host to understand dormancy and to develop new diagnostic methods and treatments for hypnozoite infections. For these studies we have developed an in vitro liver stage culture system for *P. cynomolgi*, as well as technology to genetically modify the parasite.



Research Areas
Malaria

Understanding hypnozoite biology



Using genetically modified P. cynomolgi that expresses fluorescent markers throughout the life cycle, we have been able to isolate single malaria-infected liver cells (either hypnozoites or developing liver stages) from in vitro cultures. In collaboration with Radboud University, funded by a ZoNMw TOP grant, we have sequenced RNA from these parasites and performed epigenetic analysis. Subsequent bioinformatic analysis showed the difference between sleeping and growing liver stage parasites. We identified a small group of proteins with RNA-binding motifs, exclusively expressed in hypnozoites. The genes are under epigenetic regulation and shut off during liver stage development. We hypothesize that the RNA-binding proteins prevent certain RNA's in hypnozoites to be translated into proteins, and that silencing of the RNA-binding proteins results in expression of those proteins that are essential for liver stage parasite growth. With these data, we have been able to obtain an ERC Synergy grant (Host4Hypnozoites) together with Radboud University and AmsterdamUMC, to further study the role of RNA-binding proteins in liver stage development/dormancy, as well as interactions of hypnozoites with the host liver cells.

Research Areas

Neurobiology & Aging

In 2023 the department Neurobiology & Aging further expanded the non-human primate brain bank and continued their research on aging and dementia, and further explored the effects of SARS-CoV-2 infections on the brain.

Background

Aging & Neuroinflammation – role in neurodegenerative diseases?

Advancing age is the main risk factor for the prevalent diseases of developed countries, including neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Neurodegenerative diseases progressively affect the function and structure of the brain and nervous system, leading to cognitive decline and functional impairment. These diseases are often chronic, disabling, and incurable, and can significantly impact the quality of life of affected individuals and their families.

There has been a significant increase in the incidence of neurodegenerative diseases in recent years, particularly in developed countries. This increase can be attributed to several factors, including aging of the population, changes in lifestyle, and improved diagnostic capabilities. As life expectancy continues to rise, the prevalence of neurodegenerative diseases is expected to increase further, putting a significant strain on healthcare systems and society as a whole.

Aging is not linked to a single cellular process that can explain the origin of all age-related diseases. However, over the past decade it has become more likely that age is a modifiable risk factor for disease.

One of the processes linked to aging, as well as a range of neurological disorders is **neuroinflammation**, a type of inflammation that occurs in the brain and spinal cord in response to injury, infection, or disease. During neuroinflammation, immune cells in the

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brain, microglia and astrocytes, become activated and release pro-inflammatory molecules, such as cytokines and chemokines. These molecules trigger a cascade of events that can further recruit peripheral immune cells to the site of injury or damage. Normally, inflammation is a necessary response of the immune system to protect the body from harmful stimuli, such as pathogens or toxins. However, when inflammation becomes chronic or excessive, it can lead to tissue damage and contribute to the development or progression of various diseases, including neurological disorders. In diseases like Alzheimer's and Parkinson's disease, neuroinflammation is thought to play a role in the degeneration of neurons and progression of disease.

Although we know aging and neuroinflammation are key contributors to the progression and aggravation of neurodegenerative diseases, it is challenging to determine their exact relationship. By analysing brain samples of different species and different ages, and combining it with their blood factors, including proteins and immune cells, we aim to get a deeper understanding of how aging may contribute to neurodegenerative diseases, but also neurological disorders due to viral infections, such as SARS-CoV-2.

Non-Human Primate Brain Bank

Where in 2022 we started to collect, dissect, and store the brains of non-human primates in a structural manner, in 2023 we have also applied the dissection procedure for fresh-frozen brain tissue. We have received some funding (Impetus grants) to expand and maintain the biobank as an initiative for aging research. Besides fixed and frozen anatomically dissected brain regions, we also collect blood and CSF prior from the same animals which will allow us to integrate data from the central nervous system with peripheral and central fluid compartments.

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Neurobiology & Aging

In addition, we started to collect blood plasma from the yearly physical checks of our NHP colony. In time, this will enable us to create a longitudinal dataset to investigate aging in individual animals. Our biobank is a great source for aging research, considering the lack of healthy human brain donors. The tissues are used for research interests of the BPRC but can also be utilised by external parties for research collaborations.

During 2023, brain samples from 43 non-human primates (all 3 species at BPRC) were added to the non-human primate brain bank collection.

The brain biobank is realised from our current efforts to collect as many brains as possible from the NHPs in the BPRC. Besides normal aging brains, we also collect brains from relevant disease models of BPRC, which for 2023 meant that we expanded our collection of brain tissue from macaques infected with SARS-CoV-2. In this way we are generating a collection that contains the brains of normal aging NHPs but also brains of NHPs during disease.

With the brain biobank we aim to generate a multi-omics reference of the aging brain in non-human primates.

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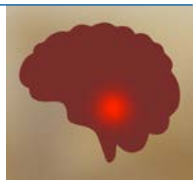
Neuroinflammation & Neuropathology in aged non-human primates



Due to the increase in the incidence of neurodegenerative disease we are investigating whether aging NHPs develop similar pathology in the central nervous system to that observed in humans. Neurodegenerative diseases are characterized by neuroinflammation and pathology in the brain, for example Alzheimer's disease is accompanied by the formation of Aβ plaques and tau tangles in the cortex.

The main focus of our research on age-related neuroinflammation is on microglia, which are the resident immune cells of the central nervous system and play a critical role in the maintenance of brain homeostasis and are the main driving force of inflammatory processes. Microglia can vary in morphology, gene expression, and response to stimuli depending on their location within the brain, developmental stage, and disease state. Understanding the heterogeneity of microglia is important for developing targeted therapies for neurological disorders and improving our understanding of the complex interactions between microglia and other cells in the brain. In 2023 we have analysed the morphology of microglia throughout the lifespan in NHPs. We have set up a pipeline for microglial morphology analysis to analyse many different parameters regarding the complexity and the shape of microglial cells. We find changes in microglia during the aging process that correlate with morphological parameters, associated with more activated microglia. With these findings we hope to identify biological hallmarks of microglia that are driving the aging process and of those that are beneficial for brain homeostasis and brain aging. Additionally, we are also investigating the presence and infiltration of the peripheral immune system in the brain.

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Neurobiology & Aging

To establish whether our NHPs show early hallmarks of neurodegenerative processes, we have started to investigate if common neuropathological hallmarks can be found in aging macaques. Preliminary data shows that with increasing age our non-human primates develop neuropathology similar to humans. Our current efforts are focusing on investigating the link between changes in the immune system and neuroinflammation with age-related neuropathology.

Neuroinflammation & Neuropathology post-COVID

Coronavirus disease 2019 (COVID-19) patients initially develop respiratory symptoms, but they may also suffer from neurological symptoms. People with long-lasting effects after acute infections with SARS-CoV-2, i.e., post-COVID syndrome or long COVID, may experience a variety of neurological manifestations. Although we do not fully understand how SARS-CoV-2 affects the brain, neuroinflammation and neuropathology likely play a role.

In 2022 we published a paper on our first SARS-CoV-2 infection study (Philippens et al., Viruses 2022), in which we describe neuroinflammatory processes in the brains of four cynomolgus and four rhesus macaques infected with the Alpha variant of SARS-CoV-2.

We continued to investigate the effects of SARS-CoV-2 infection on the brain in four rhesus macaques that were infected with the Delta variant. Besides postmortem analysis for neuroinflammation, these animals also received PET-CT scans pre-infection and at multiple time points post infection. A PET-tracer was used to image neuroinflammation, namely [^{18}F] DPA-714, which targets the translocator protein TSPO. This study revealed an increased TSPO tracer uptake throughout the brain of all infected animals already from 2 days post infection until approximately 30 days post infection, suggesting neuroinflammation is an active and continuous process even weeks after the acute infection period. Postmortem

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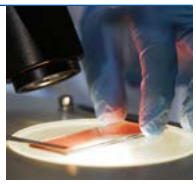
Neurobiology & Aging

immunohistochemical analysis showed increased TSPO expression and a clear response of the glial cells in various brain regions of SARS-CoV-2 infected animals. These results were published in July 2023.

Read more >

We are currently further exploring SARS-CoV-2 tropism, the mechanism behind these neuroinflammatory processes, and the neurodegenerative consequences. So far, distinct changes in glial cells have been observed in various brain regions associated with Alzheimer's and Parkinson's disease. We have also observed signs of neuronal loss, already 7 weeks post infection, which we are currently investigating further, also at time points longer after infection. This research will give us more insight on the mechanisms behind neurological symptoms seen in post-COVID syndrome and possible long-term neurological consequences.

Ageing and disease-related blood factors in humans and NHPs



Aging comes with a lot of changes throughout the body, of which many leave an imprint in the blood composition. We aim to identify how the blood composition changes with age in humans, how this may play a role in age-related diseases such as dementia, and if similar processes occur in our non-human primates.

First, we integrated the results of four human plasma proteomic datasets and compared age-associated changes of ~5000 proteins in the plasma of individuals between the age of 16 and 100. Across these datasets, we identified a set of Aging Proteins (APs) which were found to be highly associated with various age-related diseases, such as organ failure and dementia. Moreover, the expression levels of these APs in the plasma were found to be

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good predictors of chronological age, even across independent datasets. However, some individuals showed a discrepancy between their chronological age and estimated biological age based on their plasma proteome. To further investigate this difference, we identified several candidate proteins that may contribute to the acceleration or inhibition of the aging process. These results are published in Frontiers of Aging (Coenen et al., February, 2023).

Read more >

Currently, we are translating and validating our findings in the Rhesus Macaque (*Macaca mulatta*). We performed proteomics using two different platforms (SomaScan and untargeted Mass Spectrometry) on plasma samples from our own macaque colony spanning a broad age range. We identified similar associations with age in the macaques compared to several human APs. Furthermore, we are looking into shared underlying biological processes across the two species to further validate the Rhesus Macaque as a translational model of human aging. To gain a better understanding of brain aging, we selected human APs most likely reflective of this process as follow-up candidates to compare transcriptomic and proteomic changes in brain tissue. Using this approach, we aim to gain a better mechanistic understanding of brain aging, which may ultimately lead to novel therapies to remain healthy and cognitively fit for as long as possible.

In 2023, we further expanded our research to disease-related blood factors in both human and NHPs. For the latter, we are reusing in-house generated data from our NHP health checks. We are currently investigating how we can apply this data in a practical way to refine our caretaking and highlight predictors of unhealthy aging to further improve the quality of the aging process for our NHPs. Moreover, this dataset provides a unique longitudinal insight in the aging process whereas most aging studies are cross-sectional, allowing for more refined predictions in modelling aging trajectories.

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Neurobiology & Aging

For our human disease-related blood factors, we are currently looking into blood proteins associated with dementia, neuropathology and cognition. These novel biomarkers will aid in understanding human brain aging and age-associated cognitive decline. Together with our work on aging, this will ultimately lead to a selection for follow up *in-vitro* studies in which we will assess their effects on different cell types of the nervous system.

Outreach

Biomedical research is not a goal. Our goal is to understand diseases and find a cure. We cannot do that alone. That is why we share our results and discuss them with other scientists. Together we know more and that brings us closer to the solution.

We (co-)authored

39 scientific publications in peer reviewed journals.

Find the complete list here.

we gave lectures

to train students

at Dutch universities and HBOs on possibilities and restrictions of working with monkeys in research.

We actively

feed our website

with relevant updates.

We organized

bachelor, master & PhD courses

for future scientists.

During (online)

(inter)national meetings

we present and discuss our results.

We also organize

popular scientific lectures

in understandable Dutch for all BPRC staff.

We active participate in

meetings discussing alternatives

for animal experimentation.

We supervise and train

PhD, master & bachelor students.

After graduation they apply their knowledge elsewhere and contribute to science.

As partner in various research networks

our work is publicly available.

Biomedical Primate
Research Centre