BASR
2020

Biomedical Primate Research Centre
Annual Scientific Report 2020

Welcome

Join our journey through health research and alternatives

Biomedical Primate Research Centre

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Monkey Research

- Explained -

Welcome to the 2020 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 2020 BPRC housed 1277 monkeys, 824 rhesus macaques (*Macaca mulatta*), 261 cynomolgus monkeys (long-tailed macaques; *Macaca fascicularis*) and 192 common marmosets (*Callithrix jacchus*). On December 31st 2020 BPRC housed 1055 animals, 677 rhesus macaques, 234 long-tailed macaques and 144 common marmosets. In 2020 BPRC worked with 200 animals, 155 rhesus macaques, 20 long-tailed macaques and 25 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?

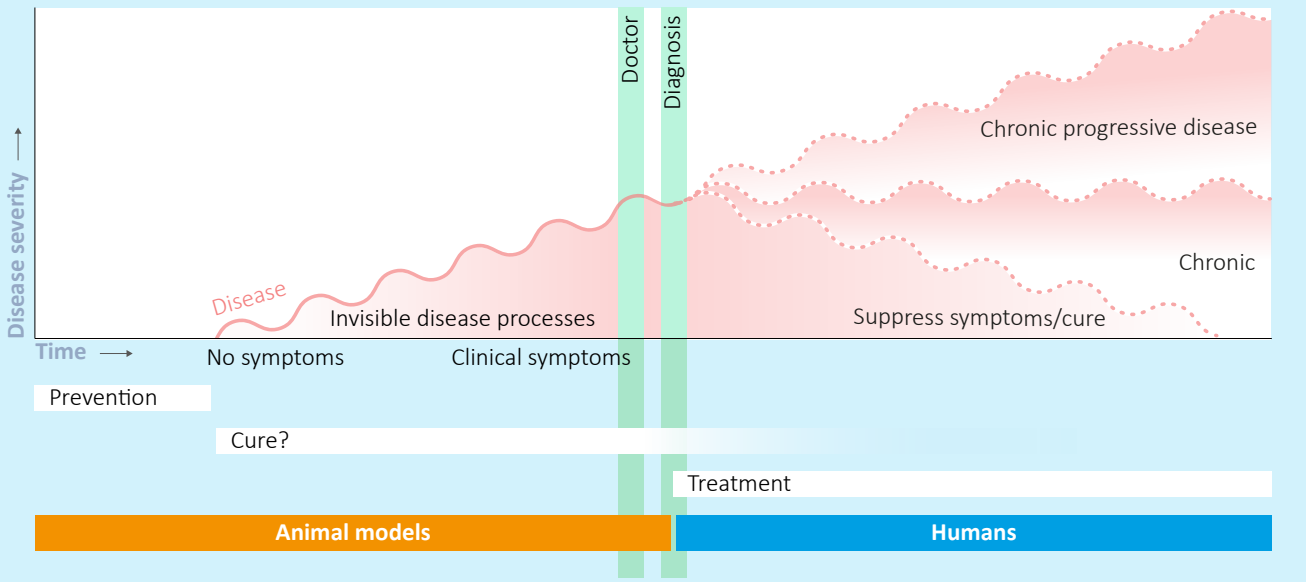
BPRC focuses on life threatening and/or debilitating diseases that affect millions of people. Diseases without cure or treatment because the complicated disease mechanisms are not yet fully understood. The European Commission concluded that the type of research conducted at BPRC cannot be done without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



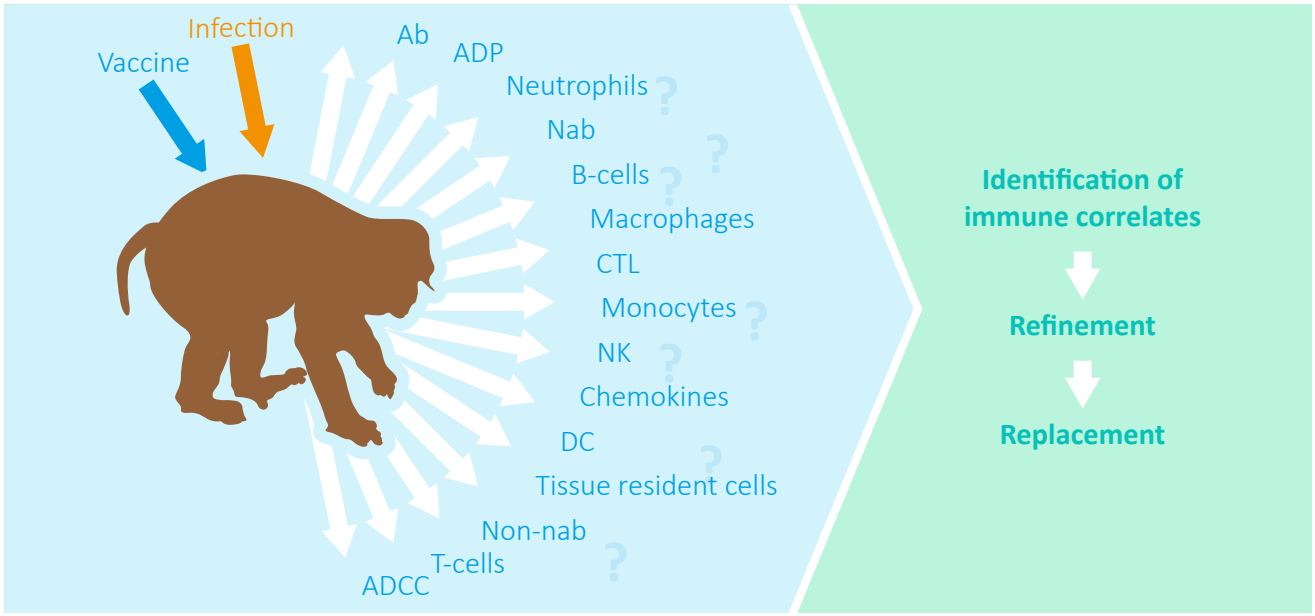
The X-axis represents the time and y-axis disease severity. A disease does typically not start with clinical symptoms. The onset is often without signs. But as time progresses the damage to cells and organs accumulate and cause clinically relevant disease. Depending on the disease this can take days 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.

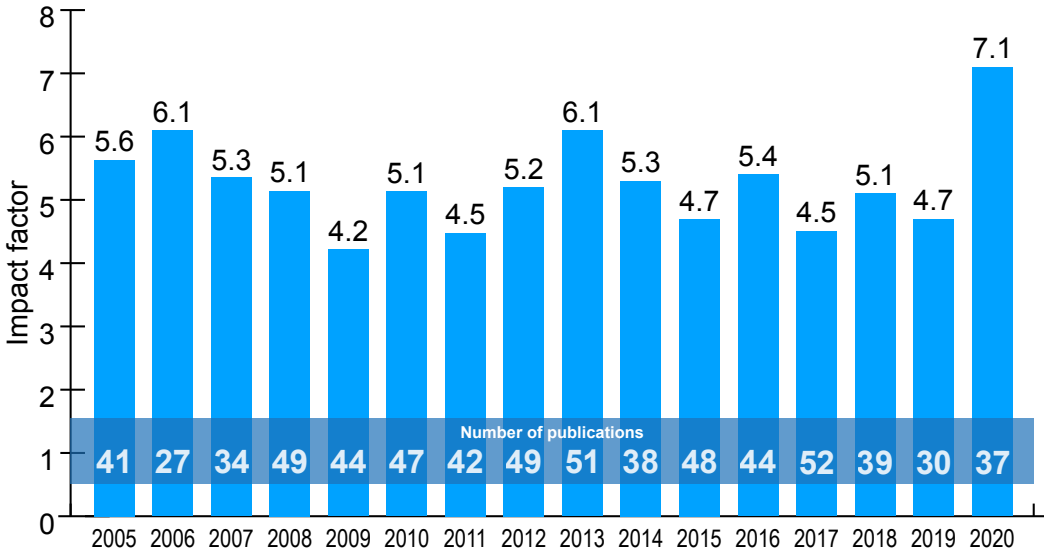
In this report we proudly present our contribution to science, the 3Rs and the development of animal-free alternatives.

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.

Message from the board

For BPRC, 2020 was another eventful year that was marked by the corona pandemic. The quality of our scientific output improves steadily, and BPRC-researchers published a substantial number of important papers in high-ranking journals. The average impact factor of these 37 scientific communications peaked at 7.09. We are very pleased with this achievement.



As one would expect, in 2020 our scientific staff performed many studies focusing on COVID-19. However we have not neglected our responsibilities in the research fields of other emerging diseases, as well as comparative genetics, animal welfare and research dedicated to alternatives. The scientific papers that are published by our staff are always highlighted at the news section of our website (www.bprc.nl). This is done in layman's language so that everybody can appreciate its content.

Message from the board

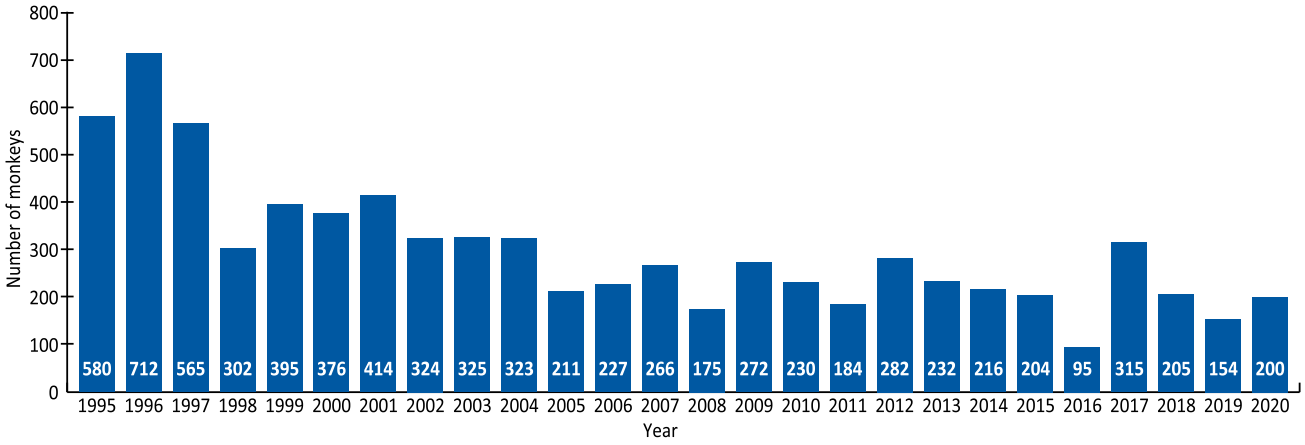
BPRC is sponsored for a large part by the Ministry of OCW. Based on our ambition plan that was approved by the ministry we have started to make some transitions. At 31st December 2020, the colony counted approximately 1000 individuals. In 2020, BPRC conducted 200 animal experiments, which represents a substantial rise as compared to 2019. This effect can be partly explained by the pandemic as BPRC is an essential institute in the quest to select safe vaccines to curb the COVID-19 pandemic. There is a world-wide need for animals to test the safety and efficacy of COVID-19 vaccines.

The other part of the explanation is due to the “counting systematics” of animal experiments in the Netherlands as defined by Dutch law. In more detail, an animal in experiment is “counted” at the end of an experiment. In 2019 some experiments were started that passed the boundary of the year and finished in2020. For that reason, BPRC prefers to works with average numbers per 5 years, which provides a more accurate estimate and the highs and lows are flattened. One can indeed see in the graphic representation below, that we have years with numbers deviating substantially from the mean. Again, this is most often explained by the length of the studies, surpassing the boundary of the year. Anyway, the trend is that the numbers tend to get lower.

After 2024, BPRC will have to fulfill the criteria set by the government that we will maximize the number of animal experiments at 120-150 a year. We are on track of achieving this, although COVID-19 research has resulted in a (temporary) increase. Most of the work that was done in the this year of the pandemic relates to the first generation of COVID-19 vaccines. It is anticipated that in the near future there will be a new wave of research concentrating on a second generation of vaccines and vaccine strategies. Preclinical research in animal models will pave the path before these vaccines can go to the clinic.

One of our great achievements was that one of the vaccine candidates that was tested in the BPRC, is now used on a large scale to vaccinate individuals all over the world.

Message from the board



The Supervisory Board and the Director congratulate the scientific and supporting staff on the obtained results.

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

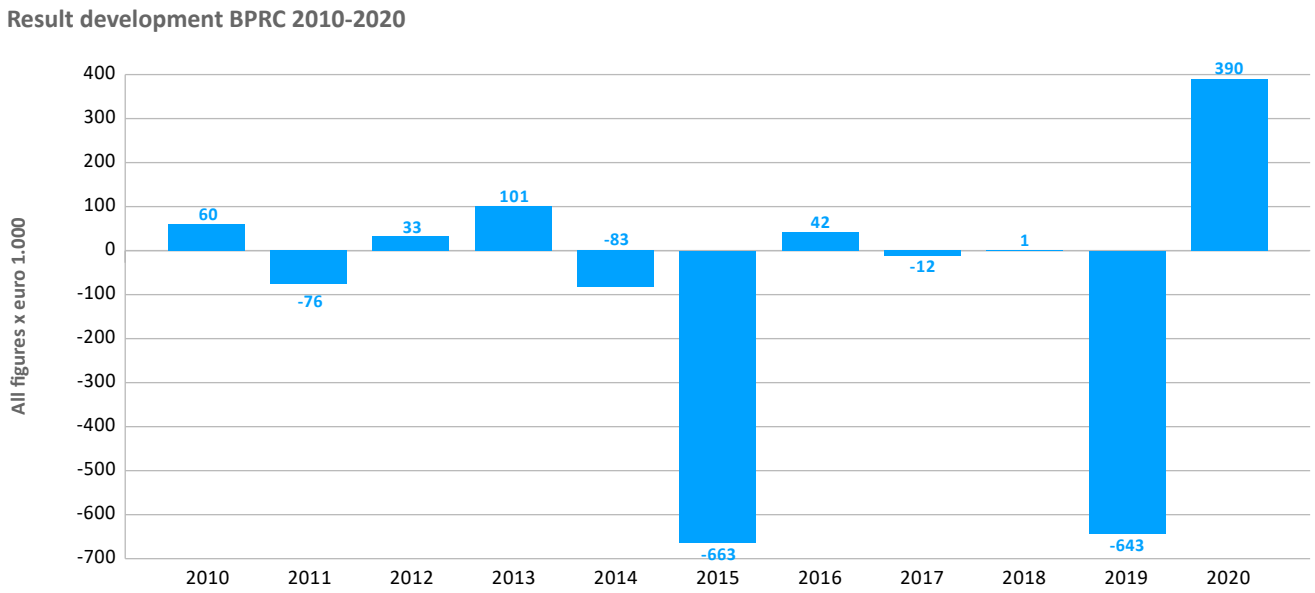
Mr. J. Vrolijk
Chairman of the Supervisory Board

Our Financial Results

Foundation Biomedical Primate Research Centre (BPRC) closed the fiscal year 2020 with a positive result of 390 K€.

Total turnover projects increased from 11,8 million euros in 2019 to 13,1 million euros in 2020, a difference of 1,3 million euros. The increase in income commissioned by third parties is caused by the COVID-19 pandemic, which has led to many new research assignments for the BPRC.

The total operating costs are in line with the budget for the year 2020.



Our Financial Results

	2020 (K€)	2019 (K€)
Turnover projects (extern)	4.812	4.324
Turnover projects (subsidy)	8.059	7.066
Total turnover projects	12.871	11.390
Other excluding interest	266	417
	266	417
Total turnover	13.137	11.807
External direct project costs	830	655
Staff costs	7.748	7.869
Depreciation	648	613
Other operating charges	3.501	3.316
Total operating costs	12.727	12.453
Profit/loss on ordinary activities	410	646-
Interest	19-	3
Profit for the financial year	391	643-
Tax	-	-
Profit for the financial year after tax	391	643-

EFFECTIVE PERSONNEL

	2020		2019	
Service Departments	16,3	16%	15,6	15%
Animal Sience Department	41,3	42%	46,0	43%
Research	41,8	42%	44,6	42%
Total	99,4	100%	106,2	100%

	2020 (K€)		2019 (K€)	
ASSETS				
FIXED ASSETS				
Buildings and structures	27.249		28.936	
Tangible fixed assets	1.780		1.439	
		29.029		30.375
CURRENT ASSETS				
STOCKS		87		47
DEBTORS DUE WITHIN ONE YEAR				
Work in progress	1.025		428	
Receivables from contracts	540		486	
Receivables tax	18		16	
Other receivables	97		874	
		1.680		1.804
Cash at bank and in hand		15.229		13.578
Total assets		46.025		45.804

	2019 (K€)		2018 (K€)	
LIABILITIES				
EQUITY				
Equity	4.117		4.760	
Revaluation reserve buildings	5.906		6.425	
Result current year	391		643-	
		10.414		10.542
PROVISIONS				
Primates	-		-	
Deferred tax liabilities	-		-	
(Flexibel) retirement	-		-	
Repairs buidings	1.872		2.240	
		1.872		2.240
LONG TERM DEBTS				
Bank	20.236		21.319	
Received in advance on asstes	6.512		7.167	
		26.748		28.486
SHORT TERM DEBTS				
Received in advance on projects	2.500		985	
Received in advance on assets	323		181	
Received in advance subsidy	908		129	
Accounts Payable (TAX)	414		457	
(Flexibel) retirement	-		-	
Accounts Payable	723		798	
Commitment Bank	1.083		1.031	
Other liabilities	1.040		955	
		6.991		4.536
Total liabilities		46.025		45.804

Our Financial Results

WNT-Verantwoording Organisatie BPRC

BEZOLDIGING TOPFUNCTIONARISSEN

Leidinggevende topfunctionarissen

Gegevens 2020	
Bedragen X 1€	
Functie(s)	Directeur
Aanvang en einde functievervulling in 2020	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	157.564
Beloningen betaalbaar op termijn	36.215
Subtotaal	193.779
Individueel toepasselijk bezoldigingsmaximum	201.000
Onverschuldigd betaald en nog niet terugontvangen bedrag	NVT
Bezoldiging	193.779
Reden waarom de overschrijding al dan niet is toegestaan	NVT
Toelichting op de vordering wegens onverschuldigde betaling	NVT

Gegevens 2019	
Aanvang en einde functievervulling in 2019	1/1 - 31/12
Omvang dienstverband (in fte)	1,0
Dienstbetrekking	Ja
Bezoldiging	
Beloning plus belastbare onkostenvergoedingen	157.441
Beloningen betaalbaar op termijn	34.775
Subtotaal	192.216
Individueel toepasselijk bezoldigingsmaximum	194.000
Bezoldiging	192.216

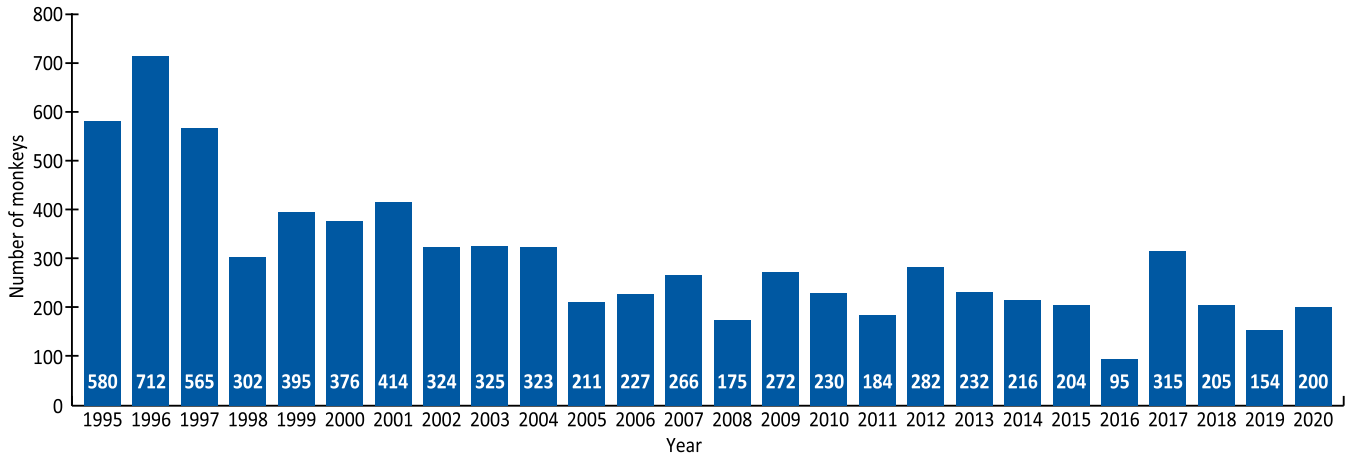
Toezichthoudende topfucntionarissen

Gegevens 2020				
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 2020	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging				
Bezoldiging	9.278	6.959	6.655	5.066
Individueel toepasselijk bezoldigingsmaximum	30.150	20.100	20.100	20.100
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 2019				
Aanvang en einde functievervulling in 2019	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12
Bezoldiging	9.278	6.959	6.655	6.755
Individueel toepasselijk bezoldigingsmaximum	29.100	19.400	19.400	19.400

Gegevens 2020			
Bedragen X 1€			
Functie(s)	Lid Raad van Toezicht	Lid Raad van Toezicht	Lid Raad van Toezicht
Aanvang en einde functievervulling in 2020	1/1 - 31/12	1/1 - 31/12	1/1 - 31/12

Facts & figures



The number of monkeys we worked with over the years and the scientific achievements in 2020.

A total of

37 scientific publications

in peer reviewed international journals.
Complete list

Three

PhD students finalized and defended

their thesis succesfully.

In addition we

train eight PhD students

to write and defend their thesis.

We performed

20,000 viral diagnostic tests

for zoos, sanctuaries and other clients.

Using

***in vitro* methodology**

we identified 12 promising compounds that prevent the development of liver stage malaria.

We placed

35 updates on our website

in the newsfeed.

We performed more than

22,000 covid tests

in the context of 10 covid studies.

We collaborated in

the first study

that reported on a NHP model for covid.

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
- 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

Energy consumption

In recent years BPRC invested in the reduction of energy consumption, for example by replacing light sources for more efficient LED-lights, replacing certain installations for more energy-efficient types and replacing the central waste container for a larger unit to reduce the required transport-movements (diesel truck) by 50% to dispose of our waste.

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 an increased metabolic activity. Increased metabolic activity can be due to cancer, infection/

Research Areas
Alternatives

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.

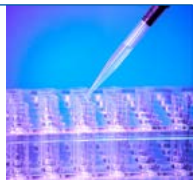
BPRC already started to use PET-CT in 2017. Initially only in our tuberculosis research but currently we are applying this state-of-the-art technique also in other research programs like influenza. With this, PET-CT is not only leading to new and more extensive scientific insights though also increasing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



Biomedical research has led to many important discoveries and new therapies, yet, some of our research affects the welfare of animals. At BPRC, we are fully aware of our responsibility to society and animals. Animals are employed for research purposes only when there are no other-alternative- methods available.

Alternative methods are categorized along the principle of the 3Rs of Refinement, reduction and replacement, all of which have a place within BPRC. When alternative methods are available, Dutch law obliges researchers to use the alternatives and forbids the use of animals. However, not many of such methods are available yet. Rather than waiting, BPRC is actively testing and developing alternative methods. The 3Rs are implemented in the research of every department,

Developing *in vitro* methods for the central nervous system

In the Western world, society is gradually aging and more and more people suffer from age-related diseases. Many of these diseases, like Alzheimer's disease, Parkinson's disease and multiple sclerosis affect the central nervous system. BPRC works with animal models for each of these diseases and aims to complement, refine, reduce and finally replace the use of animals by in vitro methods. Over the years, we have successfully developed methods to study individual brain cells using cell culture methods. In 2019 we have developed and pioneered entirely new cell culture protocols. These protocols make it possible to study cells that better resemble non-activated cells, like they are present in the normal healthy brain. This is important because many of our research questions are focused on what activates brain cells. If cells in culture are already activated, it becomes difficult to address those questions. Furthermore, we have succeeded to cultivate stem cells and to generate neuronal progenitor cells, neurons and 3D organoids. We also started a collaboration with the Technical University Delft to combine our technology with their state-of-the-art technology to develop 3D printed cell culture matrices. Read more >

Research Areas
Alternatives

European initiatives: VAC2VAC and TRANSVAC2

Vaccines are the biggest success story of biomedical science. They have changed human life expectancy dramatically. Animals are not only used during the development phase of vaccines, but also during the production and quality control phase. We participate in European initiatives that aim to reduce animal use in both phases. Vaccines are typically produced in batches. Every batch undergoes the same strict series of quality controls, involving many animal experiments, before it is released. The European VAC2VAC initiative tries to change this. The idea is that every new batch of a vaccine should not be treated as an entirely new entity, but rather as one of a series. This implies that every new batch only needs to be similar to the previous batch, possibly circumventing animal testing. To prove similarity between batches, animal-free methods are used. By adding our panel of in-house engineered cell lines (bioassays) to the consortium we characterized several different vaccines and batches in 2019. Our results demonstrate that different batches are indeed highly similar according to our tests. Together with the tests that are developed in other European labs, this initiative should lead to abandoning animal testing in vaccine batch release.

The European TRANSVAC2 initiative is stimulating innovative vaccine approaches. We contribute by making our library of bioassays available to the European research community. In 2018 we have started research on the mechanisms that affect vaccine and adjuvant efficacy when immunization is done via the skin, and this project is still ongoing.
Read more >

Developing an adjuvant without adverse effects

Adjuvants are formulations, which upon administration lead to non-specific immune stimulation. They are often used to stimulate immune responses directed against pathogens (for vaccination studies) or against components of the body itself (in animal models for human auto-immune diseases like multiple sclerosis). Some adjuvants are however notorious for their adverse effects. Most notable is complete Freund's adjuvant (CFA), which causes inflammation of the skin accompanied by granuloma formation.

Research Areas
Alternatives

Because of a lack of alternative, it is still being widely used in many animal models and also in non-human primates. Using the abovementioned bioassays, we have developed a new adjuvant in-house, MiMyc. MiMyc has been tested in a small in vivo experiment and proved to be a potent adjuvant without causing adverse effects. In 2019, we have designed a new in vivo experiment to test whether MiMyc can replace CFA in animal models for auto-immune diseases. This would represent a considerable refinement.

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.

Cooperating with friends helps monkeys relax

Stress levels of long-tailed macaques drop when they cooperate with a friend. Whereas it is known that friendship strengthens cooperative behaviour, this is one of the first studies showing an intricate way in which physiology regulates cooperation accordingly.

In collaboration with the BPRC, researchers from the University of Vienna in Austria, Utrecht University in the Netherlands have investigated the link between cooperation, social relationships and stress in long-tailed macaques. Cortisol levels, an indicator of stress, decrease when the monkeys cooperate with a befriended group member. Fourteen long-tailed macaques, living in one large monkey group at the Biomedical Primate Research Centre in the Netherlands, were trained to cooperate with other group members in order to get peanuts. Before and after the monkeys worked together, they voluntarily chewed on a saliva swab and as such provided saliva samples for the analysis of the hormone cortisol, a marker of stress. The study showed that after the monkeys had cooperated with friends their cortisol levels dropped. However, this was neither the case when the friend was just present without performing a task together nor when cooperating with a neutral individual. This rules out that cortisol levels decrease due to the mere presence of a befriended monkey, and highlights the importance of close social bonds.

Read more >

Research Areas
Ethology

High-ranking long-tailed macaques can use both aggression and affiliation to obtain resource access

Access to limited resources may be achieved by dominance as well as by high rates of aggressive and affiliative behaviour. In collaboration with the BPRC, researchers from biology and social sciences from the Utrecht University investigated the relative effectiveness of dominance rank and aggressive and affiliative behaviour in accessing three material and three social resources. Aggressive and affiliative behaviour of 24 female long-tailed macaques was scored along with their success in resource access. High-ranking individuals have more access to resources than low-ranking ones through their employment of both aggressive and affiliative behaviour. Physical aggression was effective in accessing two material resources (food and enrichment). Affiliative behaviour was effective in accessing one material (co-drinking) and one social (tolerance) resource. In conclusion, since aggressive behaviour was effective in accessing two material resources, while affiliative behaviour increased access to both a material and a social resource, affiliative behaviour is at least as important as aggressive behaviour for high-ranking individuals to access resources.

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.

In 2020, the challenge to improve the health and welfare of the monkeys in our self-sustaining breeding colonies and research facility required even more dedication and expertise than before due to the impact of the pandemic with SARS-CoV-2. Despite the new challenges of COVID-19, the animal caretakers, veterinarian staff, ethologists, laboratory staff and experts in genetics all worked together to achieve these goals.

In 2020, our macaque breeding colonies underwent major changes. The high demand for experimental animals for SARS-CoV-2 protocols, gave us the opportunity to make changes in the rhesus breeding colony. The majority of specific virus-positive animals (SIV, herpes B, SRV, STLV) in the breeding groups were selected for these experiments, and were moved to the research facility. As a result, the rhesus breeding colony is specific virus-free in 2021.

In order to achieve a virus-free colony of long-tailed macaques, several of the breeding groups were split. The adult animals were moved to a different building and the virus-free animals stayed behind. In these virus-free groups unfamiliar males were introduced to start virus-free breeding groups.

To monitor the welfare of the animals, the colony management team is observing the animals to obtain information about the stability of the groups, such as agonistic, affiliative and sexual behaviour. Despite all these changes the impact on the animals was acceptable and their welfare was not compromised.

Besides their assistance and input in the welfare of the animals in our colony, our ethology department is also involved in applied and fundamental behavioural research. In this field, Astrid Rox successfully defended her thesis in Utrecht in 2020 after some delays due to COVID-19.

Research Areas
Tuberculosis

Tuberculosis (TB) is a bacterial infection that causes lung disease. TB is one of the deadliest infectious disease by a single pathogen in the world. In 2020 1.4 million died of TB. Only to be surpassed by the number of people who died of COVID (1.9 million in 2020). In addition, over 10 million people fell ill from TB and approximately 25% of the world population is latently infected with TB. In Western countries TB patients are treated with a cocktail of antibiotics. However, anti-microbial drug resistance in TB is increasing, 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 the only means to break the cycle of TB transmission and infection.

Read more >

BPRC uses TB models in the rhesus monkey and the cynomolgus monkey to get a better insight in how the disease develops and to evaluate new treatments of TB. The two models represent two different disease manifestations. TB in rhesus monkeys develops as a progressive active form of the disease, while infection of cynomolgus monkeys can develop as a latent disease. Combined these models best recapitulate the full manifestations of tuberculosis in human.

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 was 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’

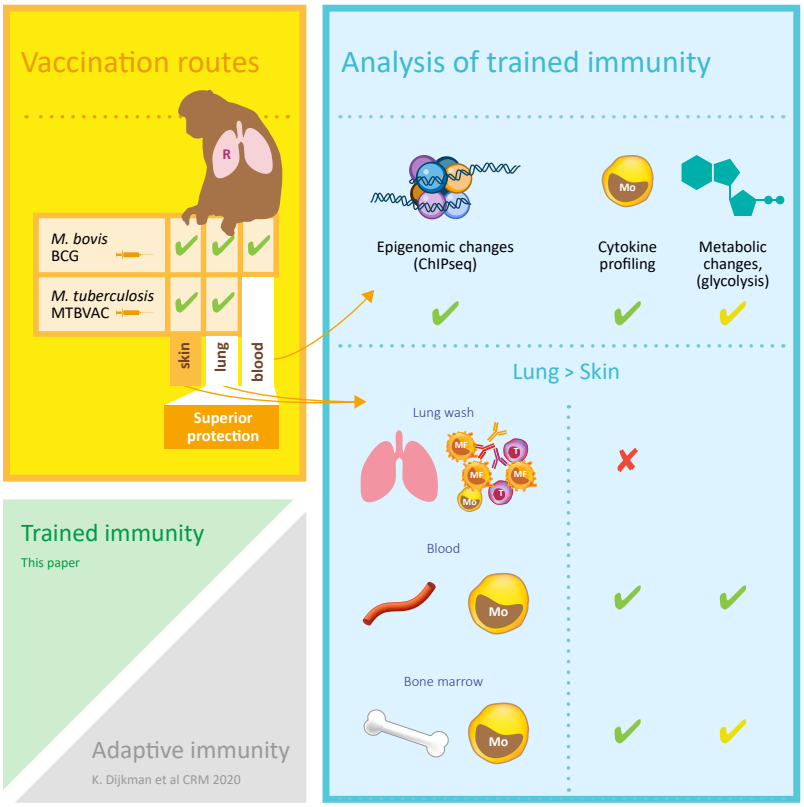
Research Areas
Tuberculosis

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 protection against other respiratory infections.

We previously have 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.

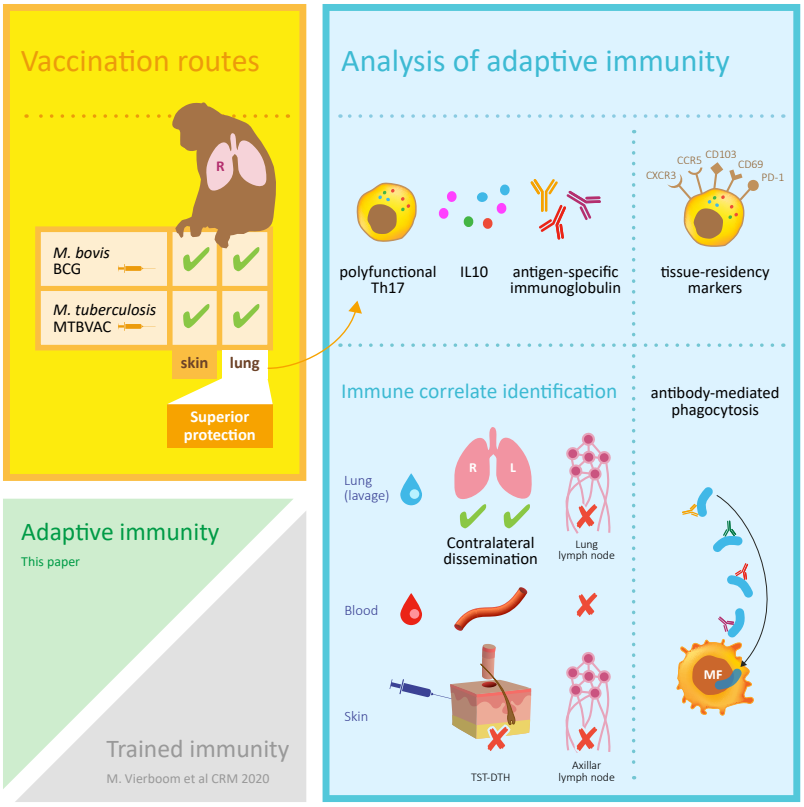
In 2 recent studies, that were reported in Cell Reports Medicine, we extended our analyses of the immune response observed after mucosal vaccination with BCG.



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

Read more >

In addition, we analyzed the new vaccine candidate MTBVAC that was derived form a human clinical isolate of mycobacterium which is different from BCG which is derived from bovine mycobacterium.



We demonstrated that mucosal vaccination with MTBVAC also displays the same improved induction of trained immunity combined with a broadened immune response and a similar adaptive immune signature associated with the improved protection after mucosal BCG vaccination.

Read more >

These results provide support to strategies for improving TB vaccination and, more broadly, modulating innate immunity via mucosal surfaces.

Research Areas

Comparative Genetics & Refinement

The immune system is orchestrated by many different genes. These genes can differ from individual to individual, and this genetic variation is called polymorphism. Polymorphisms explain why some people are susceptible for a certain disease while others may be resistant to development of the same disease. Hence, the diversity generated by genetic polymorphisms prevents the elimination of an entire population by one single pathogen.

The killer cell immunoglobulin-like receptor (KIR) and leucocyte immunoglobulin-like receptor (LILR) system, which are located in the leucocyte receptor complex (LRC), and the major histocompatibility complex (MHC) are examples of polymorphic gene systems. A successful immune response is multifactorial, and depends, for instance, on the cooperation between the KIR and MHC system. In general, the MHC system is involved in discriminating between self and non-self and thus the recognition of invading pathogens while the KIR and LILR systems may be seen as fine tuning and serve as a correction mechanism for the MHC system. KIR genes are involved in the immune defence to viruses and cancer cells, while LILR genes play a role as modulators of infection and immunity and are engaged in neural function.

Monkeys are genetically similar to humans. Understanding genetic polymorphisms in monkeys, and their role in the immune system, teaches us much about the functional immune defence in humans. This is particularly important in the development of a whole new generation of medicines, the so-called personalized medicines.

In the Comparative Genetics and Refinement department, we investigate MHC, KIR, and LILR genes from different monkey and ape species. For this, we use DNA-sequencing and other techniques, like fragment analyses on short tandem repeats (STR). We not only study the DNA from animals from our own breeding colonies but also DNA samples from other institutions and zoos.

Research Areas

Comparative Genetics & Refinement

Comparative analysis of *KIR* genes in two different macaque species revealed rapid evolution

The outcome of infections and immune responses in preclinical macaque models might be affected by the selection for specific macaque species and even for certain geographical populations. A differential response is, for instance, demonstrated upon SIV infection in rhesus macaques originating from the Indian and Chinese populations. The diverse outcomes are most likely explained by genetic variation.

The killer cell Ig-like receptors (KIR) modulate NK cell education and activity through interactions with MHC class I molecules; two receptor families that are encoded by the most complex gene clusters in primates. Using transcriptome sequencing, we defined the KIR gene content of rhesus and cynomolgus macaques, and their different populations. Many new genes were defined that were mostly generated by chromosomal recombination, and included KIR genes that were either shared between species or species-specific. Even more, high levels of polymorphism were recorded, but only a few alleles were shared in species and populations. The genetic variation at KIR gene and allele level in two closely related macaque species and their populations demonstrate an unparalleled rapid evolution and selection.

These findings pave the way to study the impact of different KIR repertoires in macaque models for human health and disease. In addition, knowledge on the variability of KIR genes may help in selecting animals with particular genetic markers to refine experiments.

Read more >

Read more >

Research Areas

Comparative Genetics & Refinement

Complex tale of conservation, diversification and inactivation: Comparative genetics of HLA-F and its orthologues in different primate species

HLA-F is one of the three non-classical MHC class I molecules. Where classical MHC class I molecules (named HLA-A,-B,-C) are expressed on all nucleated cells, characterized by high polymorphism and involved in antigen presentation to T-cells, non-classical MHC class I molecules show low levels of polymorphism, have a restricted tissue distribution, and interact in general, but not exclusively, with receptors of the innate immune system. Only recently some clues on the function of HLA-F were brought to light.

The presence of *HLA-F*-like genes have been identified in several evolutionary distantly related non-human primate species. To get a broader view on the diversity and polymorphism present at the population level, we have recently characterized *HLA-F*-like genes in a large population of chimpanzees, rhesus and cynomolgus macaques, and marmosets, and several individuals of gorilla's, orangutans, pig-tailed macaques, baboons, grey-bellied night-monkeys and cotton-top tamarins. These analyses revealed that also in different non-human primate species *Mhc-F* shows low levels of polymorphism. In most species only one active *F*-like gene is seem to be present on a chromosome, like observed in humans. An exception is formed by the marmoset, where the gene has been subject to duplication, and active and inactive genes can be found on a chromosome.

With this comparative analysis, in which we used solely tissue bank derived samples, we have enlarged our knowledge on evolutionary ancestry of the non-classical MHC-F molecule, which is now accessible for the research community. In future times it has to be sorted out whether these HLA-F orthologs in different primate species also execute similar function.

Read more >

Research Areas

Comparative Genetics & Refinement

Humans, chimpanzees and bonobos share MHC-A molecules with similar functional characteristics: implications for controlling of retroviral infections

Chimpanzees and bonobos are humans closest living relative, which is reflected, for instance, in a highly similar immune system. However, one of the differences between these species can be found in the way they respond to an HIV-1 infection. Most humans that get HIV-1-infected develop AIDS. Chimpanzees that get infected with HIV-1 or an HIV-1-like virus are on the other hand relative resistant to developing AIDS. In bonobos, HIV-1-like infections appear to be absent.

A minority of humans can naturally control an HIV-1 infection, and particular MHC molecules have been shown to play a role in this. A while ago, we have demonstrated that chimpanzees and bonobos experienced a selective sweep that targeted their MHC class I repertoire, which was most likely caused by a retroviral infection, such as HIV-1. Therefore, characterization of the relevant MHCs in our closest living relatives might reveal clues how their immune repertoire was shaped.

By using samples from our Biobank and computer modelling, we recently have investigated the functional characteristics of MHC-A molecules in chimpanzees and bonobos. These analyses revealed that humans, chimpanzees and bonobos share MHC-A molecules that can function in a similar way. The abundant presence of precisely this type of molecules suggest that they might play a role in controlling retroviral infections in these species.

Read more >

Read more >

Research Areas

SARS-CoV-2 and COVID-19

Last year, the world was startled by SARS-CoV-2, the causative agent of COVID-19. The department of Virology started a new research line to combat the pandemic.

No animal-free alternative

Starting a new research line in monkeys, why? Why not start directly with innovative animal-free techniques? The rationale behind this is very simple, as animal free methods that enable animal-free testing of vaccines are still miles away. That is not so much our opinion, but also the opinion of Stichting Proefdiervrij, a Dutch foundation that promotes the development of animal-free research.
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 infects macaques and causes pneumonia

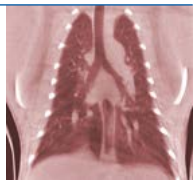
BPRC co-authored the first paper that showed macaques are indeed susceptible to SARS-CoV-2. Experimental exposure led to virus excretion from the nose and throat, and postmortem examination showed pathology in the lungs. On April 1st 2020, the Centrale Commissie Dierproeven approved BPRCs request to use macaques for SARS-CoV-2 research. The approval comprised the development of non-human primate models, and the evaluation of vaccines and antiviral drugs.

From experimental infection to animal model

Knowing macaques can be infected with SARS-CoV-2 is a good start but much needs to be done before we can actually speak of an animal model. Detailed information is needed on how the infection progresses in vivo, and how you can recognize and monitor disease. For a good animal model it is also important to have specific, optimized and standardized laboratory assays to monitor infection and immune responses.

Research Areas

SARS-CoV-2 and COVID-19



The 3Rs, Refinement-Reduction-Replacement

Setting up a new animal model is a good opportunity to include new innovations and techniques to obtain more data from an animal study. Apart from the implementation of standard 3R measures, like group housing and training, we are exploring 24/7 real-time measurement of body temperature and physical activity. CT (anatomical information) and PET-CT (combining anatomical and functional information) imaging is used to visualize infection and disease. Optimization of nose and trachea swabbing was also done. Many questions are still unanswered. Do we need bronchoalveolar lavages to quantify virus in the lungs? This technique is more invasive but also provides more information than nose and throat swabs, for instance because you can study immune cells from the lungs. Also important, does this type of sampling affect the course of infection?

At the same time laboratory tests needed to be developed, optimized and standardized, like RT-qPCR, ELISA, neutralization assays, flowcytometry and many others.

Research Areas

SARS-CoV-2 and COVID-19

SARS-CoV-2 in macaques

In 2020, the department of Virology performed several SARS-CoV-2 infection studies to develop the model. Similar to what other research groups found, SARS-CoV-2 typically did not cause severe pulmonary disease in macaques. We observed transient shedding virus from nose and throat, and occasionally from the gastrointestinal tract. The animals did not develop high fever and little or no changes in blood chemistry or hematology were seen. After approximately 15 days, we could measure IgG antibodies in the blood, implying the immune system had recognized and responded to the virus. Medical imaging techniques showed mild to moderate lesions in the lungs, and at postmortem analysis we saw enlarged lymph nodes residing near the lungs also indicating that the virus had interacted with the immune system.

Vaccines and antiviral drugs

In the course of the year 2020, the department of Virology tested 10 vaccine candidates and one antiviral drug. Several manuscripts describing that work are currently in preparation and are expected to be available in 2021.

Research Areas

Influenza virus

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

Respiratory Viruses

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. In order 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, we studied whether non-human primates could be infected by virus in aerosols, The animals indeed became infected, but the reaction of the body was somewhat different than 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 of an 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.

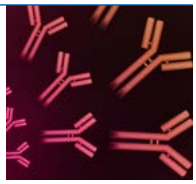
Read more >

Research Areas

Respiratory Viruses

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.



PhD student influenza specific antibody responses

Protection from influenza infection relies on good antibody responses. In collaboration with the Amsterdam Medical Center we are currently training a PhD student to study the plasticity of antibody-producing B-cells. For this project we use *in vitro* models, and blood samples from previously vaccinated rhesus macaques. The results of the project will lead to better understanding of antibodies after influenza-vaccination.

Research Areas
HIV-AIDS

AIDS is caused by an infection of the Human Immunodeficiency Virus (HIV). Antiviral medications are able to delay HIV growth and can prevent AIDS development. Furthermore, these compounds are able to 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 continuous. This year we have started a new so called prime-boost vaccination strategy as part of a large EU consortium. The study will continue into 2021.

Research Areas

Mosquito-borne Diseases

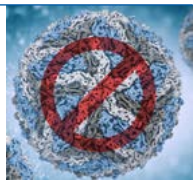
Dengue virus, West Nile virus, Rift Valley fever virus, and Zika virus are mosquito-borne viruses that cause an infection in people. In most people, the infection is transient and without clinically relevant illness. However, approximately 1% of the patients suffer from complications. Nowadays, over 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.

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 medicine 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 hospitalisation and about 20,000 people die of severe dengue.

Previously, we collaborated with a European partner in a preclinical study to evaluate an antiviral drug. The results of this proof of concept-study were promising. The treated animals had less virus in their blood compared to the non-treated control animals. To investigate the broader spectrum of this drug new infection models were needed. In 2020, the department of Virology further invested in these models.

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

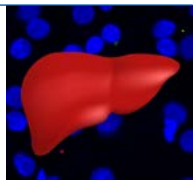
Mosquito-borne Diseases

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 commercialised 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. A manuscript describing the results is presently in preparation.

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 dormant parasite stages in the liver, called hypnozoites. Hypnozoites are only formed in 5 primate malarias (including 2 human malarias) and we use *P. cynomolgi*, that infects rhesus monkeys, as a model for *P. vivax* to study hypnozoites and discover new drugs that are urgently needed to kill hypnozoites. For this we have developed an *in vitro* liver stage culture system for *P. cynomolgi.*



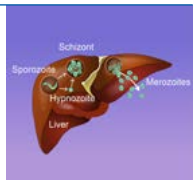
Improving drug screening for dormant malaria parasites

In 2019, we screened a small preselected chemical compound library of over 500 compounds for activity on *P. cynomolgi* liver stages, including hypnozoites. While data are still being analysed and confirmed, we found some 8% of the compounds having activity against developing and/or dormant liver stages. In a large collaborative study, we contributed to the development of an *in vitro* culture system for *P. cynomolgi* blood stage parasites. Although the parasite strain used may not be particularly suited for liver stage research, this work raises hope that in the future we may not need to infect monkeys anymore to supply us with blood stage parasites for further studies. In another collaboration we identified a protein, expressed during the liver stages and marking the point when liver stage parasites start to grow. Thus, this protein is not expressed in hypnozoites. We are currently using this information to develop new parasite lines that differentially express different enzymes in hypnozoites and growing liver stages. Such parasites will be used to develop enzymatic read out in more optimal *in vitro* drug assays.

Read more >

The grant under which the *in vitro* drug assays were developed has been extended to set-up a new collaboration for implementing a fully automated, medium – high throughput in vitro screening of new compounds against dormant liver stages of malaria exploiting the enzymatic read-out.

Research Areas
Malaria



Detecting the presence of dormant malaria parasites

Currently there is no diagnostic test to detect hypnozoites in the human population. Such a diagnostic test would be very useful at the time we want to wipe out hypnozoites from the population with new drugs, because it would identify individuals that need treatment. Together with Japanese colleagues, using our *in vitro P. cynomolgi* hypnozoite culture, we are working to pioneer development of a diagnostic test. In large scale experiments, covering many different variables, we identified a promising signature of molecules that seem to signal that host liver cells are infected with hypnozoites. The next step is to determine whether these molecules can also be detected in a hypnozoite-infected monkey.

In the beginning of 2020 a funding request was submitted to the GHIT Fund to support the next step in the project. The request was approved in mid 2020 and in the end of 2020 the preparations for the project's execution were carried out so that the project could be carried out in 2021.

Research Areas

Neurodegenerative diseases

Western societies are facing an increasing prevalence of age-related neurological diseases caused by progressive degeneration of the brain. This development is a concern for the ageing Western societies, as 80% of the elderly population suffers from at least one chronic disease. There is still no cure for most of these diseases, such as Alzheimer's and Parkinson's disease.

Therapies for Alzheimer's and Parkinson's disease are still based on symptom control treatment. Understanding the underlying mechanisms represents the most essential research effort in neurosciences to find targets for treatment to cure or prevent these diseases. The lack of relevant translational animal models that faithfully reproduce clinical and pathogenic features is a major cause of the delay in finding useful targets for therapy development.

BPRC developed models for neurodegenerative diseases to learn more about the underlying mechanisms and to validate new treatment strategies to limit disease progression and improve patients' quality of life..

Deep brain stimulation for cognitive improvement in Parkinson's Disease

Parkinson's Disease (PD) is caused by the death of neurons in a part of the brain that controls movement. This damage results in the typical motoric symptoms. Besides motor disfunction, patients also suffer from dementia. The cognitive decline is one of the most disabling symptoms of PD. This type of dementia is called PD dementia (PDD).

Recently, a PD model has also been validated in the larger rhesus monkey for improving cognition by deep brain stimulation (DBS). In this new model, the cognitive decline in PD is modeled by a localized brain lesion in the nucleus basalis of Meynert (NBM) that controls cognition through the innervation of the cerebral cortex with a messenger compound,

Research Areas

Neurodegenerative diseases

acetylcholine. The NBM is also damaged in PD patients suffering from PD dementia. The localization for the lesion in the NBM is determined using MRI and PET-CT brain scans. The cognitive changes of memory and learning are tested in the home cage using a touchscreen tablet. The monkeys learn to use the tablet to perform cognitive tasks. Correct performance is rewarded with random images of a landscape on the tablet and a food reward. Once this model is established, a DBS device can be placed in the monkey in the same way as in PD patients. However, the outcome of this treatment in patients is not stable due to the type of stimulation related to stage of the disease. Currently, DBS consist of a chronic stimulation frequency. In the model we will compare different stimulation algorithms, chronic low frequency, intermittent, and based on the original firing of the cells in the brain. The positive outcome will be compared with the level of damage in the brain corresponding to the different stages of the disease.

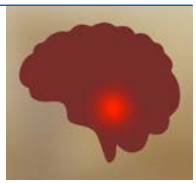
PD research at BPRC is focused on understanding the processes that contributes to the pathology and clinical expression of the disease. Our models are used for the development of pharmaceutical and non-pharmaceutical therapies that stop the neurodegenerative process, suppress disease symptoms and prevent side effects of the current medications.

Read more >

Research Areas

Neurodegenerative diseases

Identifying features of neurodegeneration



Alzheimer's Disease (AD) is a brain disease that slowly affects memory and the ability to carry out simple tasks of daily living. About 50-80% of all cases of dementia are caused by AD. As cells die over time, affected brain regions begin to shrink. This degeneration seems to be woven by aggregation of abnormally folded proteins called amyloid plaques and tau tangles that are causally linked to cellular stress and inflammation in the brain.

An inflammatory response may well be responsible for this progressive spread of abnormally folded proteins throughout the brain. These aggregations of proteins hinder communication in the brain and finally resulting in disconnections, brain atrophy and memory loss.

At the BPRC brains are collected from monkeys that have died for other reasons other than experimental studies. From this brain bank, brains of monkeys ranging from 1 to 26 years old have been examined for markers for misfolded proteins and for immune-related changes such as activated microglial cells. Over time, the brain expresses cells related to an aging immune system. These changes go hand in hand with the presence of Alzheimer's plaque development. This ongoing study offers the opportunity to link different processes in the brain during aging, which will help to learn more about the dynamics of this disease and to find targets for treatment.

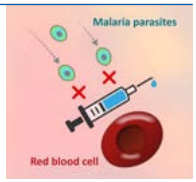
Research Areas

Protein Core Facility

BPRC is often a collaborative partner to evaluate new vaccine candidates. Yet, we also design vaccines ourselves. Scientists at BPRC have many years of experience in vaccine development.

COVID vaccine

BPRC is in the process to produce an attenuated virus vaccine against COVID19. Using Transformation Assisted Recombination techniques, hybrid common cold-SARS CoV2 coronaviruses are being constructed, among others with the delta variant of the virus. When available, these will be tested for safety and efficacy as a potential superior vaccine. Moreover, they may serve as research tools to elucidate the mechanisms by which monkeys, and also humans, protect themselves against COVID.



Malaria vaccine

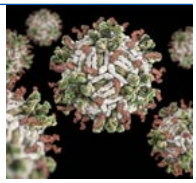
PfAMA1, a protein vaccine against the deadly malaria parasite *Plasmodium falciparum* was developed at BPRC's protein core facility. AMA1 plays a key role in the entry of the parasite into red blood cells and therefore AMA1 is a good vaccine candidate. After optimizing vaccine design, expression and purification procedures the vaccine was used in a proof-of-concept study in monkeys. After an extended vaccination procedure PfAMA1 provided partial protection against the malaria disease. However, in people the vaccine did not suffice. Combining PfAMA1 with another vaccine candidate may overcome this in the future.

Research Areas

Protein Core Facility

The AMA1 protein from the second-most important human malaria, *Plasmodium vivax*, has also been produced and is being tested as a potential vaccine. First results show that high levels of antibodies are induced after vaccination that prevent invasion in red blood cells in functionality tests in the lab. Tests in humans are intended, but external funding needs to be acquired for this purpose.

Virus like particles vaccine platform



Virus-like particles (VLPs) are molecules that resemble viruses but lack the virus' genetic material, and thus are non-infectious. VLPs are capable of activating the immune system and generate a virus-specific immune response. Therefore, VLPs are sometimes used as a vaccine. The vaccine that is currently used to protect women from cervical cancer caused by HPV is also based in virus like particle technique.

In line with this, BPRC's protein core facility designed VLP-vaccines for Usutu virus and Zika virus. Both vaccine candidates are in early stage of development. The envelope proteins were produced by recombinant techniques, purified and biochemically prepared to generate VLP's. Vaccination studies showed that antibodies obtained after vaccination with usutu or ZIka protein fractions prevent virus replication in functionality tests in the lab. However, the proteins need to be adapted to optimize VLP formation. After that, they will be further evaluated for immunogenicity and their potential as human vaccines.

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

37 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 were visited by Algemeen Dagblad

for the recording of a documentary

on the role and necessity of monkey research in the development of vaccines and treatments against SARS-CoV-2.

We actively

feed our website

with relevant updates.

We organized

bachelor, master & PhD courses

for future scientists.

During

(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

via TransVac2, HONOURs, Vac2Vac, Aeras, and Bill and Melinda Gates Foundation CTVD.