MSc Infection & Immunity

Appendix 2

Description of research themes

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Infection

In spite of the advent of antibiotics, antivirals and numerous successes in the combat of infectious diseases in the past century it has now become painfully clear that known and newly emerging infectious diseases will be a major challenge for the world at large in the coming decades. Research focuses on interactions between infectious pathogens and the host. This happens with significant collaboration between the respective working groups, taking advantage of complementary research activities and infrastructure of other research programs within Erasmus MC. The long-term goal is to limit the clinical, epidemiological and economic impact of infections, using state of the art technologies.

Immunity

Research focuses on the search into the key molecular processes regulating the proliferation and differentiation of immune cells. The basic aspects of the program are complemented by research components related to the function and dysfunction and deficiency of the differentiated “end” cells both in physiological conditions and in disease (e.g. granulopenia, immunodeficiencies, autoimmunity). Specific programs have an extension towards clinical application and involve investigations related to developmental diagnostics and therapeutics (e.g. molecular diagnostics, pharmacogenetics). Thus the program covers a spectrum from basic towards clinically applied investigations.

Infection & Immunity

Fundamental knowledge of immunology and microbiology lays the foundation for the development of novel diagnostic strategies and the development of therapeutic strategies. The research programs are solidly embedded in and interacting with investigators, scientific groups and networks in a broad international context (e.g. cooperative clinical trial groups, European task forces, scientific groups). This holds both for the laboratory parts and the clinical activities. The strong clinical outlet of the program directly determines the societal impact of novel clinically introduced strategies. Researchers within the program have leading positions in international clinical and research networks and hold leading advisory positions. The program yields as a spin off various patent applications.

The program is centered on several integrating main working areas:

- *Viral infections*
  This working group focuses on acute respiratory virus infections, chronic virus infections and infections with herpes viruses. Studies on the natural and vaccine-induced immune response to pathogenic viruses are performed to improve existing and develop novel vaccines. Knowledge of the (immuno-) pathogenesis of these virus infections, either in humans or animals, forms the fundamental basis of this work. Depending on the viral pathogen and whether humoral or cellular immunity is desired, subunit vaccines, whole inactivated vaccines, live-attenuated vaccines or vectored vaccines are studied. The efficacy of novel and existing antiviral treatments and the emergence of drug-resistant virus variants are monitored to improve antiviral treatment regimens. The discovery of novel viruses associated with human diseases is included in this workgroup since the etiology of many human and animal diseases is still unknown; the identification of viral pathogens may lead to development of vaccines and antivirals to limit their clinical impact.
- *Molecular pathogenesis and epidemiology of infectious diseases*
  Molecular pathogenesis and epidemiological studies on *M. mycetomatis, M. catarrhalis* and *S. aureus* infections are carried out using state of the art technology like the screening of expression

libraries and SNP analysis. In addition the molecular identification and characterization of *C. jejuni* strains with or without GBS association are subject of research.

- *Pediatric infectious diseases and immunology*
  Interactions between the host and pathogenic bacteria - *Mycoplasma pneumoniae, Streptococcus pneumoniae, Neisseria meningitidis*, coagulase-negative staphylococci and *Moraxella catarrhalis* - are studied at the molecular level to improve diagnostic, therapeutic and preventive modalities.
- *Gastro-enterology; Helicobacter pylori*
  Three separate research areas are addressed: characterization of the role of metal- and acid-responsive regulatory networks in the pathogenesis of Helicobacter infection, both in gastric and enterohepatic Helicobacter species; fonding of a national expertise centre for the diagnosis, prevalence and resistance testing of H. pylori; exploitation of different Helicobacter species as a model system for the study of inflammatory enteric (IBD) and hepatic diseases (HCC).
- *Pediatric gastro-enterology; mucosal responses to bugs and drugs*
  Mechanisms underlying normal function and disorders of the gastrointestinal tract in childhood and adult life are studied by means of integrated pre-clinical and clinical research. This research aims at the development of strategies for prevention, diagnosis, and treatment of gastrointestinal diseases. Specific attention is focused on the mucosal damage and repair, and on the cross talk between luminal agents (e.g. bacteria, viruses) and the gastrointestinal epithelium, and on the cross talk between the epithelium and the underlying immune cells.
- *Liver immunology*
  Liver Immunology research within the Erasmus MC has a strongly translational character with basic scientists and clinicians working closely together. The basic research is performed by a rapidly growing group of scientists from the Department of Gastroenterology and Hepatology and the Department of Surgery. In order to form a coherent group, all basic scientists are situated in the Laboratory of Gastroenterology and Hepatology. Basic and clinical research is tightly integrated with basic scientists and clinical researchers from both departments having weekly research meetings together.
- *Transplantation immunology*
  The main topics studied are: cytokines and chemokines in organ transplantation; pharmacotherapy and pharmacogenetics in organ transplantation; immune tolerance and infection after liver transplantation; and ischemia reperfusion injury.
- *Mucosal immunology*
  In the laboratory of the Department of Pulmonary Medicine we focus on basic and translational research in the field of lung inflammation in asthma, COPD and pneumonia. By studying patients and mouse models we aim (1) to elucidate the role of dendritic cells in directing and maintaining a chronic localized immune response to the lung, and (2) to identify the molecular mechanisms responsible for the humoral immune response, which is essential for host defence against bacterial pathogens, but defective in asthma and COPD.
- *Lymphoid differentiation and immunodeficiencies*
  The research aims of this program comprise to study the role of lymphoid-specific transcription factors that control the in vivo developmental program of lymphoid cells, to study signal transduction routes that are crucial to stem cell self-renewal and differentiation into lymphoid cells, to unravel the essential steps in the induction and execution of Ig/TCR gene rearrangements, to characterize the signal transduction pathways that are downstream of the antigen receptors and are essential for survival, selection and developmental progression of lymphoid cells, to investigate how defects in the regulation of differentiation and proliferation steps during lymphoid development result in immunodeficiencies, and to translate the obtained knowledge on normal lymphoid differentiation and gene defects into novel diagnostics and opportunities for gene therapy in patients with primary immunodeficiencies (PID).
- *Transplantation and genetic modification of hematopoietic stem cells*
  Manipulation of the immunosystem is necessary for successful clinical organ transplantation. This may be achieved by prescribing immunosuppressive regimen, allowing engraftment that is traded with debilitating comorbidity associated with aspecific immunosuppression. Success may also be accomplished by tapering the immunosuppresive load allowing the emergence of immunological countermechanisms leading to non-responsiveness. In the setting of clinical organ transplantation

we study donor specific alloreactivity in an attempt to understand the immunological pathways leading to success or failure. Our specific aim is to find optimal therapeutic strategies for the individual patient as well as to elucidate the role of cytokines/chemokines in the cascade of events that lead to acute rejection, graft dysfunction, and graft acceptance after clinical transplantation under conditions of immunosuppression.

- *Immune regulation and autoimmunity*
  Chronic inflammation and autoimmune disease are leading causes of morbidity, psychosiocal burden and economic loss in Western society. In view of the central role of the innate and adaptive immune system in these diseases, detailed insight into immune regulation is a requirement for rational development of diagnosis and (immuno) therapy.

Viral infections

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Goals of research: general outline

The long-term goal of this working group is to limit the clinical, epidemiological and economical impact of virus infections by vaccination and treatment with antiviral drugs. This working group focusses on acute respiratory virus infections (influenza viruses, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), measles virus (MV)) and chronic virus infections (hepatitis B and C viruses, lentiviruses (HIV-1, HIV-2), herpes viruses (herpes simplex virus (HSV), varizella zoster virus (VZV), and phocid herpes virus (PHV)). Studies on the natural and vaccine-induced immune response to pathogenic viruses are performed to improve existing and develop novel vaccines. Knowledge of the (immuno-) pathogenesis of these virus infections, either in humans or animals, forms the fundamental basis of this work. Depending on the viral pathogen and wether humoral or cellular immunity is desired, subunit vaccines, whole inactivated vaccines, live-attenuated vaccines or vectored vaccines are studied. Vaccines are produced using classical techniques or state-of-the-art techniques in biochemistry and molecular biology. Humoral and cellular immunological parameters are monitored, with a special emphasis on vaccine-induced immunopathogenesis for selected viral pathogens. The efficacy of novel and existing antiviral treatments and the emergence of drug-resistant virus variants are monitored in order to improve antiviral treatment regimens. Our high quality molecular diagnostics unit forms an important bridge between clinic and research. The efficacy of antiviral drugs to combat AIDS (HIV-1, HIV-2) and hepatitis (hepatitis B and C viruses) is measured in relation to drug-resistance, host parameters and epidemiological parameters. A novel HIV vaccine is evaluated for the treatment and prevention of AIDS. The discovery of novel viruses associated with human diseases is included in this workgroup since the etiology of many human and animal diseases is still unknown; the identification of viral pathogens may lead to development of vaccines and antivirals to limit their clinical impact.

Scientific achievements during the last 5 years

Influenza

Identification on H5N1 influenza as the cause of fatal disease in humans in Hongkong.
Identification of swine H1N1 and H3N2 viruses as the cause of serious disease in humans.
Identification of influenza B virus seals.
Identification of a novel influenza A virus subtype; H16.
Identification of H7N7 viruses in poultry and humans.
Generation of an up-to-date HA/NA database through surveillance studies in wildlife.
Provided insight in the role of cellular immunity in influenza virus infections.
Provided insight in the role of cytokine responses in influenza virus infections.
Evaluated the use of ISCOMS as candidate influenza vaccines.
Evaluated the effect of bioactive compounds on immunity to influenza viruses.
Set up primate models to study influenza A virus (H5N1, H3N2) infections.

Set up reverse genetics system for influenza A virus.
Detailed quantitative analyses of influenza virus drift.
Studies on molecular determinants of influenza virus pathogenesis.

Human metapneumovirus
First identification and characterisation of this novel human respiratory pathogen.
Established clinical/epidemiological impact.
Identified 4 genetic lineages representing 2 serotypes.
Set up animal model systems.
Set up reverse genetics systems.
Designed and evaluated vaccine candidates
Studies on innate immunity
Studies on virus host range

Respiratory syncytial virus
Generated animal model to study immuno-pathogenesis induced by RSV vaccines.
Provided insights in the immunopathogensis associated with formaline inactivated vaccine.
Evaluation of the safety of BBG2Na, a recombinant RSV subunit vaccine.
Evaluation of safety and immunogenicity of a recombinant MVA vaccine.
Evaluation of TH-1/TH-2 responses in relation to disease severity.
Evaluation of RSV subgroups/genetic lineages in relation to disease severity.
Characterisation of CTL responses upon RSV infection.
Development of Genomics technology to study respiratory virus infections.

Measles virus
Generated an animal model to test measles vaccines.
Evaluated MVA vectors to be used as measles vaccine.
Evaluated the use of ISCOMs as measles vaccine.
Evaluated pulmonary and neurological safety of aerosol-administration of live-attenuated vaccine.
Evaluated diagnostic and molecular epidemiologic techniques for measles virus in Sudan.
Genotyping of measles viruses from Sudan.
Comprehensive analysis of antibody responses to wild-type MV infections (Sudan).
Studies on imported cases of measles in The Netherlands.

Hepatitis viruses
Demonstration of selection of HBV mutants during lamivudine therapy of hepatitis B.
Developed tests to detect HBV genomes by real time PCR.
Developed techniques to quantitate HBV ccc DNA, an intermediate in the replication cycle of HBV.
Identification of new hepatitis C variants.
Characterisation of hepatitis B viruses from non-human primates (gibbons).
Analysis of cytokine responses by different cell types.
Analysis of host factors during antiviral therapy of hepatitis C patients.
Analysis of the role of CTLs in the response and relapse upon interferon therapy.
Modification of mathematical models for virus population dynamics based on clinical parameters.

Lentiviruses
Definition and identification of parameters critical for CTL-efficacy in controlling HIV reproduction.
Generated animal models.
Development of a new vaccination strategy for the treatment and prevention of AIDS.
Inventarization of HIV-2 infected persons in Rotterdam. Built up of a cohort and technology for the study of HIV-2.
Characterization of HIV-2 isolates.
In particular for HIV-2, technologies have been developed and implemented to determine the viral dynamics of replication in patients during antiviral treatment.

Herpes viruses

Insight in the dynamics of viral populations in bone marrow transplant recipients, resulting in new intervention strategies.
Demonstration and detailed characterization of the systemic/intra-ocular HSV-specific B and T cell responses in patients with HSV-induced stromal keratitis and uveitis.
Development and application of a PCR-based assay to genotype HSV-1 isolates revealing risk factors for the development of HSV-induced keratitis and uveitis.
Identification of the putative role of corneal resident cells in the immunopathogenesis of HSV-induced stromal keratitis.
Demonstration and characterization of the systemic/intra-ocular VZV-specific B and T cell responses in patients with VZV-induced uveitis.
Demonstration of two variants of PHV-2 with different tropism.
Demonstration of oncogenic properties of PHV-2.

Coronaviruses

Identification of a novel human coronavirus, HCoV-NL63.
Participated in theWHO-coordinated SARS-aetiology network.
Generated initial genome sequences of SARS coronavirus.
Demonstration that SARS-CoV fulfilled Koch's postulates as causative agent of SARS.
Development of animal models for SARS.
Demonstration that IFNs could be used as antiviral treatment for SARS.
Development of SARS vaccine candidates and testing in animal models.

Virus discovery

In addition to the newly discovered viruses listed above, several viral pathogens have been discovered in the more distant past. Recently, and not listed above, we have been involved in he identification of a circovirus in wild birds, an orthoreovirus in budgerigars, , and morbilliviruses in seals.

Future plans: special goals and approach

For the respiratory virus infections, we will continue to perform surveillance studies in humans and animals in order to study genetic and antigenic differences between virus isolates, which is important for vaccine development. This work will be performed within our tasks as WHO National Influenza Center, partially funded by E.U. framework 6 and 7 programmes, the NWO Nivarec programme, and collaborations with industry. We will continue to develop novel vaccines and test vaccines developed by others using our available animal model systems. It is anticipated that some of these vaccines will be tested in clinical trials within the next 1 to 5 years, at least in part sponsored by pharmaceutical industries. Fundamental research on virus properties is essential for our understanding of viral pathogenesis, the immune responses required to control virus infections, identification of the viral targets most suitable for vaccine development and our understanding of immunopathogenesis associated with vaccination. Such projects are currently mostly funded by fundamental research foundations (N.W.O., E.U, and NIH.) and new projects have recently been approved or are under evaluation.
The large genomics project (VIRGO) includes studies on immunopathogenesis related to RSV and measles virus vaccines, pathogenic influenza virus infections, and (enhanced) disease models for RSV, measles, influenza and human metapneumovirus.

For the chronic virus infections, the current line of work on characterisation of emerging (drug-resistant) viruses will be continued, funded in part by the E.U. and pharmaceutical companies. This work is done in collaboration with clinical departments of Erasmus MC and elsewhere, and relies heavily on the molecular diagnostic unit of the department of Virology. Data gathered within this working group will be used to suggest improvements in current antiviral therapy regimens and to modify existing mathematical models. Virus-host interactions that are critical in relation to disease progression and therapy-efficacy will be studied, again sponsored by the E.U. and pharmaceutical companies. The role of CTL in controling disease, and the correlation of cellular immunity with therapy efficacies will be further evaluated. For the herpes viruses, studies on immunopathogenesis and virus properties have

recently been initiated and will be continued. A Phase I/II clinical trial in humans with a tat/rev-based vaccine to treat and prevent AIDS will be initiated.
The virus discovery work will be continued and expanded through collaborations with other departments of Erasmus MC (neurology, pediatrics, internal medicine) to identify novel pathogens associated with human disease. These projects will first by funded internally, but upon identification of novel pathogens will likely be funded by pharmaceutical industry.
This working group has extensive contacts with pharmaceutical industry (Solvay pharmaceuticals, Roche, GSK, MedImmune, Numico, Isconova, GSF, Biomerieux), has been succesful in obtaining grants in The Netherlands, Europe and elsewhere, and has a strong track-record in IP protection and the generation of succesful spin-off companies (most recently ViroClinics B.V. and ViroNovative B.V.). The working group thus has formed a strong basis to continue to secure its financial situation.

10 most important publications

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10. **Verjans GM**, **Hintzen RQ**, van Dun JM, Poot A, Milikan JC, **Laman JD**, Langerak, AW, Kinchington PR, **Osterhaus AD**. Selective retention of herpes simplex virus-specific T cells in latently infected human trigeminal ganglia. Proc Natl Acad Sci U S A 2007 104:3496-501. **IF 9.6**

Molecular pathogenesis and epidemiology of infectious diseases

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Goals of research: general outline

Molecular Pathogenesis

M. catarrhalis is a human-specific respiratory microbial pathogen also responsible for a large fraction of otitis media cases in children. With the increasing success of vaccination against *Haemophilus influenzae* and pneumococci, the leading causes of otitis media, the infectious burden of *M. catarrhalis* is foreseen to drastically increase over the coming years. It has been shown that complement-resistant *M. catarrhalis* is more virulent that the susceptible counterparts. Our research aims are twofold. First we study the molecular basis of complement resistance and, second, we aim to develop a vaccine for the prevention of infection. To achieve these goals we have developed an autologous transformation

system, performed high-throughput comparative genomics for a wide (phenotypic) variety of strains and developed proteomics approaches for identification of surface proteins, putatively involved in microbial pathogenesis.
S. aureus persistently colonises the nasal epithelial linings of approximately 1 in 3 humans. Another similarly sized fraction of humans is incidentally colonised whereas the remaining people never carry *S. aureus*. Our main research focus is to elucidate the molecular mechanisms of nasal carriage. We performed several large-scope microbiological culture-based surveillance studies. These involved 3000 elderly people, 3000 children in the age of 1-18 yrs and several smaller groups. These carriage studies helped define the Dutch *S. aureus* carriage incidence figures. We performed longitudinal follow up studies, performed clinical placebo controlled intervention studies and are involved in nasal inoculation of mixtures of *S. aureus* strains in volunteers. We recently discovered that *S. aureus* colonisation is not a neutral process. It seems that *S. aureus* indices a local, mild inflammatory process.
M. mycetomatis causes madura in sub-saharan Africa. These infections are characterised by massive lesions, usually in the extremities. We have been developing an animal model for the analysis of fungal pathogenicity pathways. In addition, the availability of the animal model will facilitate therapeutic studies and the identification of host susceptibility factors.

Epidemiology
With the help of molecular typing we have been able to demonstrate for otitis media that there is a schism between complement resistant and susceptible isolates to an extend suggesting the existence of two species rather than two variant of a single species. High-throughput comparative genomics has now resulted in numerous genetic markers for the two putative species.
Staphylococcus aureus, frequently colonising the nasal epithelial linings, is the most frequently used model organism for our genetic studies. We are involved in several large-scale surveillance studies involving thousands of volunteers. Genetic comparison of isolates thus gathered has revealed that in the Rotterdam area six major clonal types of *S. aureus* circulate, all but one able to induce infections. We also performed longitudinal follow up studies in single individuals for periods over 8 years in duration. The methicillin resistant variant of *S. aureus*, so-called MRSA is a very-hard-to-treat variant. Its clinical importance has initiated a multitude of studies on its international dissemination. We have been tracking MRSA clones with new and old technologies in diverse countries.

Infectious diseases, campylobacter jejuni and GBS
The objective of the current research is to describe the molecular epidemiology and elucidate the molecular and immunological mechanisms leading from an infection with *C. jejuni* to the development of GBS. Central to this collaborative project is the molecular mimicry hypothesis, i.e. that the host immune response towards bacterial LOS results in cross-reactive antibodies against human gangliosides. The current project involves a combination of sophisticated chemical and physical analysis of bacterial carbohydrates, combined with site-directed mutagenesis of the bacterial species involved, complemented by frontier sensor technology and animal disease models. Ultimately, the project aims to define the epitopes involved in the molecular mimicry causing a cross-reactive immune response, to resolve the mechanism of an aberrant immune-response, and to generate novel diagnostic tests to identify pathogens that have an increased potential for triggering auto-immune sequelae.

Scientific achievements during the last 5 years
Molecular Pathogenesis
A novel molecular detection system and an animal model of infection for *M. mycetomatis*.
We defined complement resistant *M. catarrhalis* as a specific lineage within the species; current data even suggest that a completely new bacterial species might have been discovered.
Development of an autologous transformation system for *M. catarrhalis*.
The uspA1 and uspA2 proteins are not likely to become successful vaccine molecules due to naturally occurring intra-chromosomal recombination and escape mutagenesis.
OmpA is a novel marker for complement resistance in *M. catarrhalis*, immunogenic in rabbits and a candidate vaccine molecule.
Nasal application of mupirocin in orthopedic surgery leads to a significant drop in the number of post-operational infections.

Severity of non-bullous impetigo in children depends on the virulence gene potential of the causative *S. aureus* strain.

- HNPs play an important role in the mediation of staphylococcal carriage in humans and a certain single nucleotide polymorphism in the encoding gene predisposes towards carriage.

Epidemiology

Demonstration of the genetic diversity of *Candida albicans* populations in patients infected with HIV and demonstration of the continued evolution of *C. albicans* during antifungal therapy.
Short sequence repeat polymorphism in invasive and colonising *C. albicans* strains is not associated with adaptive responses related to virulence characteristics.
Aspergillus fumigatus spores in the outside air display seasonal density variability and the spore density is associated with the risk of acquiring infections among the immunocompromised.
Complement resistant *M. catarrhalis* is a specific lineage within the species; current data even suggest that a completely new species might be discovered.
Genetic analyses have uncovered that the population structure of *M. catarrhalis* is relatively diverse with some obvious clonal expansion.
Exacerbations of lung diseases in intensive care patients are not due to epidemic dissemination of certain virulent *M. catarrhalis* genotypes.
People can carry the same *S. aureus* strain for periods spanning more than eight years.
S. aureus causes infections in cattle and the dynamics of these infections depend on various factors including usage of milking equipment, contact between farmers and cattle and genetics of the host.
Molecular typing of *S. aureus* can be standardised among different institutions in different countries facilitating data exchange and efficient tracking of MRSA clones.
MLST for *S. aureus* can be performed using DNA chip technology.

Infectious diseases, campylobacter jejuni and GBS

Molecular characterization of GBS related Campylobacter confirming diverse genetic lineages.
Molecular characterization of Campylacter in Curacao detecting specific clonal lineages.
Detection of a C.jejuni gene associated with immune-mediated neuropathy.
Molecular characterization of the LOS biosynthesis locus.
Description and association of a specific LOS biosynthesis locus class with GBS.
Induction of anti-GM1 and anti-GQ1b antibodies in rabbits by Campylobacter LOS.
Expression of well-defined ganglioside mimics on Campylobacter LOS determines antiganglioside specificity in rabbits.

Future plans: special goals and approach

Molecular Pathogenesis

In case of *M. mycetomatis* infections, answers are sought through the development and serological screening of expression libraries, SNP analysis of of DNA samples from patients and people exposed to the fungus but not overtly infected, usage of the recently developed animal model and the use of mouse genome chips to screen for gene expression modification upon infection with *M. mycetomatis.* For *M. catarrhalis* we intend to develop the protein into an adequate vaccine by production of recombinant protein. We are following up on a number of additional vaccine candidate molecules at the same time. During our *S. aureus* research we will focus on both the host and the bugs in designing strategies primarily aimed at elimination. We are currently trying to interfere in carriage by the use of *S. aureus* specific bacteriophages, and we are exploring the human factors involved in maintenance of carriage, both at the macroscopic and the microscopic level. We identify immune responses at the protein level and are on the search for molecular determinants of *S. aureus* susceptibility (genomic scanning using SNP approaches, for instance in human neutrophil peptide genes and other (innate) immune response genes). Studies in volunteers involving artificial colonisation with wild type and mutant *S. aureus* strains are a continuing effort. Support for this research line has been obtained from NWO and several other financiers.

Epidemiology
The near future will bring the development of sequence-based assays including the assessment of single nucleotide polymorphism in target genes. The detection of SNPs using high-throughput whole genome screening methodologies is currently employed and will be further developed over the coming years. We have a specific interest in those SNPs that are associated with (severity of) infections caused by staphylococci, campylobacters and *Moraxella catarrhalis*. In addition we are exploring human SNPs associated with susceptibility to infections caused by the previously mentioned microbial pathogens. We aim to develop systems where we can detect significant polymorphisms, identify genes involved and characterise microbial pathogenicity pathways by molecular dissection of the microbial genes involved. Finally, we will relentlessly pursue similar studies in the future, since there appears to be a growing intereist in both the technological aspects and the clinical implications of such studies. We are currently, for instance, instructing and collaborating with scientists from diverse countries.

Infectious diseases, campylobacter jejuni and GBS
Significant research funding has very recently been obtained by an international, multi-continental taskforce including four collaborative groups from R'dam, Wageningen, Dokkyo (Japan) and Ottawa (Canada) by the Human Fontier Research Progam 2003-2006 ($1.350.000).
Main research questions are: (1) Are there consistent genetic differences between *C. jejuni* strains from GBS patients and from enteritis only patients (without neurological symptoms)? (2) What are the exact shared epitopes on *C. jejuni* LOS and gangliosides towards which the autoantibodies are directed? Can these epitopes be synthesized *in vitro* and can these molecules be used for improved diagnostics of GBS? (3) Which factors are critical for expression of a neuro-pathogenic LOS on *C. jejuni* and are these moieties equally immunogenic in different hosts?

10 most important publications

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Pediatric Infectious Diseases and Immunology

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Goals of research: general outline

Worldwide, infectious diseases are the major cause of childhood morbidity and mortality. Primary and secondary (acquired) immunodeficiencies are increasingly diagnosed and often complicated by serious infections. The studies carried out within the research program of the Division of Pediatric Infectious Diseases and Immunology of Dr. N.G. Hartwig and Dr. C. Vink aim to increase the understanding of interactions between host and micro-organisms and to improve the diagnostic, therapeutic and preventive modalities of infectious diseases and immunological disorders. Major disease entities under study involve respiratory tract infections with *Mycoplasma pneumoniae*, neonatal infections, infections caused by *Neisseria meningitides* (meningococci) and HIV, as well as immunodeficiencies. The studies on *M. pneumoniae* and *N. meningitidis* have been initiated at the end of 2006, following the appointment of Dr. C. Vink as new head of the Laboratory of Pediatrics. The research program seeks to integrate clinical (patient-related), translational (disease-related) and basic research. Major subjects of research include the pathogenesis of infection by *M. pneumoniae* and *Neisseria meningitidis*, the host-pathogen interactions in children with viral and bacterial respiratory tract infections and HIV/AIDS, and the characterization of primary immunodeficiencies. Research of the molecular pathogenesis of *M. pneumoniae* infections focuses on the identification and characterization of bacterial genes and protein which play a role in attachment of the bacterium to the respiratory epithelium and which may undergo antigenic variation leading to evasion from the host's immune response. In addition, over the past 5 years, various studies have been undertaken to investigate the molecular epidemiological behavior of pathogenic bacteria. The predecessors of Dr. Hartwig and Dr. Vink, Prof. Dr. R. de Groot and Dr. P.W.M. Hermans, who left the Rotterdam group in 2005, have initiated these studies. Finally, a top-reference research center for children with HIV/AIDS has been established since 1998. Research in this field includes immune reconstitution, studies on viral resistance, pharmacokinetics and pharmacodynamics of antiretroviral drugs, as well as epidemiological and clinical studies.

Scientific achievements during the last 5 years

Over the last 5 years, various studies were undertaken to investigate the molecular epidemiological behavior of bacterial pathogens, in particular *Streptococcus pneumoniae*. These studies relate to the national and international spread of (multi)drug-resistant pneumococcal isolates, the epidemiological characteristics of penicillin resistance, the nosocomial transmission of multidrug-resistant isolates in the Netherlands, the epidemiological characteristics of colonization and infection, and the epidemiological impact of vaccination on pneumococcal carriage and infection. The scientific effort has resulted in a valuable collection of strains, which have been well characterized, and many publications in international, peer-reviewed journals. Research of the molecular pathogenesis of pneumococcal infections focuses on the identification and characterization of pneumococcal genes and key regulatory components, which play a role in the pathogenesis of infection. To reach these goals, a combined proteomics- and genomics-based approach is applied. Proteome and transcriptome profiles of *S. pneumoniae* are developed to identify (virulence) genes that are selectively expressed during survival of the pathogen in the host. Various virulence factors have been identified and characterized so far, and some of them are promising candidates for future vaccine design. The latter findings have been claimed in two patent applications.

The host susceptibility for and the immune response against invasive bacterial infectious diseases, in particular by meningococci and pneumococci, are studied since the early 1990s. The identification of genetic polymorphisms in immune-response genes, which influence susceptibility for and severity of invasive diseases, is currently a major focus of investigation. Research on meningococcal infections focusses on the role of coagulation factors, chemokines and cytokines in the pathogenesis of infections and on the host response to vaccination. This work has resulted in three PhD theses and over 30 international publications. A top-reference research center for children with HIV/AIDS has been established since the mid nineties. Research in this field includes immune reconstitution, molecular virology, pharmacokinetics and pharmacodynamics of antiretroviral drugs, and epidemiological and clinical studies. Pediatric HIV/AIDS research has resulted in 20-30 papers in international peer-reviewed journals. The development of a top-reference center for primary immunodeficiencies in collaboration with the Department of Immunology of Erasmus MC has also resulted in several PhD

theses and over 20 publications since 1995. Vaxinostics is a limited liability company founded by Prof.dr. R. de Groot, Dr. P.W.M Hermans and Dr. H.C. Rümke, and offers a broad spectrum of academic skills and expertise related to infectious diseases to pharmaceutical partners. The research activities within Vaxinostics have also contributed to the scientific spin-off by means of international publications. Vaxinostics has recently been transferred to Radboud University Nijmegen.

Future plans: special goals and approach

We will further expand the research program of the Division of Pediatric Infectious Diseases and Immunology, which aims to increase the understanding of interactions between host and micro-organisms and to improve the diagnostic, therapeutic and preventive modalities of infectious diseases and immunological disorders. In particular, we will extend our efforts to elucidate the molecular mechanisms that play a role in the interactions between pathogenic bacteria and the host. Fur this purpose, we will extend the functional genomics approach to elucidate these mechanisms through genomics-based grant applications. In addition, infectious diseases-oriented foundations will also be explored in future. Obviously, our research activities have clear commercial interest. Hence, biotech companies and pharmaceutical industries are most willing to participate in our line of research.

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Workgroupleaders	**Department**
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Dr. P.W.M. Hermans	Pediatrics, UMC Radboud
Dr. A. Warris	Pediatrics, UMC Radboud
	Division of Pediatric Infectious Diseases and Immunology

Goals of research: general outline

The research line on Pediatric Infectious Diseases and Immunology within the Department of Pediatrics of the Radboud University Nijmegen Medical Centre originates from the Division of Pediatric Infectious Diseases and Immunology (Dr. P.W.M. Hermans, Prof.dr. R. de Groot, and Dr. A. Warris). The division considers as one of her core priorities the organisation of top clinical and top reference care for children with serious and complicated (pediatric) infectious diseases, immune deficiencies, immune-mediated diseases and HIV/AIDS.

Scientific achievements during the last 5 years

Teaching, training and research in the fields of pediatric infectious diseases and immunological disorders in children are developed in close collaboration with top reference patient care. The research of the group has a predominantly translational profile, although clinical (patient-related) research and to a lesser extent basic research also forms part of the research profile. The research is embedded within the Nijmegen University Centre of Infectious Diseases (NUCI). The division has been recognized by the Pediatric Association of the Netherlands as a training centre for pediatric infectious diseases and immunology. There is a close collaboration with the Division of Pediatric Infectious Diseases at Erasmus MC in Rotterdam (head: Dr. N. Hartwig) in the areas of fellow training and research.
The translational research of the group is performed within the Laboratory of Pediatric Infectious Diseases (head: Dr. P.W.M. Hermans). Within the Laboratory of Pediatric Infectious Diseases the pathogenesis, immunology and epidemiology of pediatric infectious diseases are the central research themes. In close collaboration with various laboratories and medical departments within Radboud University Nijmegen Medical Centre, the Laboratory of Pediatric Infectious Diseases aims to improve the molecular and clinical understanding of the biology of infectious diseases. The laboratory research team has great experience in the growth and genetic manipulation of *S. pneumoniae*. Bacterial genomic fingerprinting, genomic array footprinting, transcriptional profiling (micro arrays, Q-PCR) and proteome profiling (two-dimensional gel electrophoresis and quantitative mass spectrometry; profiling of both pathogen and host), high-throughput protein identification (vMALDI, nano-LC LTQ-FT-ICR-MS), *S. pneumoniae* virulence - and vaccination studies in animal models for both invasive and non-invasive disease as well as asymptomatic carriage, and functional analysis of bacterial proteins are carried out within various research projects. The Laboratory of Pediatric Infectious Diseases has recently expanded its activities towards infections caused by *Moraxella catarrhalis, Haemophilus influenzae* and *Staphylococcus aureus.* In collaboration with various renowned researchers in the field of microbial pathogenesis, we seek to study the molecular interaction between host and microorganism. Our work searches for the development of tools to diagnose treat and prevent infectious diseases. Hence, our work contributes to the medical care of children suffering from life-threatening infections.

Future plans: special goals and approach

The clinical research of the division focuses on the treatment of HIV/AIDS in children in collaboration with Dr. D. Burger of the Department of Clinical Pharmacy, on fungal infections with a focus on the clinical epidemiology of pediatric fungal infections and the development of novel diagnostic tools (Dr. A. Warris), on viral respiratory tract infections (Dr. P.W.M. Hermans, Prof.dr. R. de Groot) and on immune deficiencies.
In the coming years collaboration within the Department of Pediatrics will be expanded with a focus on neonatal infections (Division of Neonatology), respiratory tract infections (Division of Pulmonology), intensive care infections (sepsis research), and infections in oncology patients (Division of Pediatric Oncology). In addition, we will focus together with the Department of Clinical Pharmacy on the clinical research of antimicrobial drugs in children. Vaxinostics has recently been transferred to Radboud University Nijmegen.

Prof.dr. R. de Groot and Dr. P.W.M. Hermans are also involved as advisors in Vaxinostics BV, a university-affiliated company, which performs vaccine studies in children and adults, and studies in the field of nutrition and immunology.

10 most important publications

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Gastro-enterology: *Helicobacter pylori*

Workgroupleaders
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Goals of research: general outline

Chronic inflammation in the stomach caused by *Helicobacter pylori* is an important factor in the development of peptic and duodenal ulcer disease, and gastric malignancies. Research aims to improve the understanding on long-term survival and adaptation of *H. pylori*, on the pathogenesis of chronic *H. pylori* infections, and on improved diagnostics and treatment of *H. pylori* infection. In addition to *H. pylori*, increasing attention is given to non-pylori Helicobacter species. Reflux-induced chronic inflammation is thought to be initiating factor of the development of Barrett's esophagus, a precursor of adenocarcinoma of the gastro-esophageal junction. This hypothesis is being tested both in patients, animal model systems as well as in vitro. Finally, the putative role of non-pylori *Helicobacter* spp. in nongastric pathologies such as hepatocellular carcinoma and Inflammatory Bowel Disease is being investigated.

Scientific achievements during the last 5 years

The urease enzyme is one of the most important virulence factors of the gastric pathogen *Helicobacter pylori*. The enzyme produces ammonia, which allows *H. pylori* to colonize the gastric mucosa by neutralization of the acidic environment. We have shown that the expression of the urease enzyme, and alternative enzymes for the production of ammonia are carefully balanced via the levels of diverse metal ions and the NikR and Fur regulators. These regulatory proteins also influence the expression of many other important metabolic systems, and thus represent possible alternative targets for the development of novel antimicrobial compounds.

Eradication of *H. pylori* from patients is known to often result in healing of *H. pylori*-associated diseases like peptic ulcers. Unfortunately the number of usable antibiotics for treatment of *H. pylori* infection is very limited, and is hampered by the development of antibiotic resistance. We have characterized the molecular mechanisms mediating resistance to all four commonly used antibiotics (metronidazole, clarithromycin, amoxicillin, tetracycline), and have developed molecular tests for the rapid screening of *H. pylori* strains for antimicrobial resistance.

Colonization of the gastric mucosa by *H. pylori* results in many changes in the mucosa, including chronic inflammation of the colonized tissue. We have investigated the interactions of *H. pylori* with

epithelial and immune cells, and have shown that *H. pylori* can modulate interleukin expression in immune cells. We have also identified possible new virulence markers of *H. pylori*, which will allow better distinctions between virulent and non-virulent strains.
It is becoming clear that like *H. pylori* in humans, many other animal species have their own specific *Helicobacter* species, in the gastric, enteric and hepatobiliary system. We have sofar focussed on the hypothesis that many gastrointestinal and hepatobiliairy cancers may be connected to yet unknown *Helicobacter* species. We have recently shown that liver samples of patients with hepatocellular carcinoma, which have none of the usual riskfactors, are significantly more infected with a *Helicobacter* species. Currently we are trying to culture this *Helicobacter.*

In addition to these patient-based studies, we also use the murine enterohepatic Helicobacter species *H. hepaticus* as a model system for human hepatic and enteric Helicobacters.

Future plans: special goals and approach

Characterization of the role of metal- and acid-responsive regulatory networks in the pathogenesis of Helicobacter infection, both in gastric and enterohepatic *Helicobacter* species.
Fonding of a national expertise centre for the diagnosis, prevalence and resistance testing of *H. pylori*.
Exploit different *Helicobacter* species as model system for the study of inflammatory enteric (IBD) and hepatic diseases (HCC).

10 most important publications

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Liver Immunology

Workgroupleaders **Department**
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Goals of research: general outline

Liver Immunology research within the Erasmus MC has a strongly translational character with basic scientists and clinicians working closely together. The basic research is performed by a rapidly growing group of scientists from the Department of Gastroenterology and Hepatology and the Department of Surgery. In order to form a coherent group, all basic scientists are situated in the Laboratory of Gastroenterology and Hepatology. Basic and clinical research is tightly integrated with basic scientists and clinical researchers from both departments having weekly research meetings together. Liver immunology is focussed on three research lines, all using similar techniques and collaborating closely together:

Viral Hepatitis.

The aim of this research line is to investigate which virus- and host related conditions are required for immunological control of hepatitis B (HBV) and hepatitis C (HCV) viruses, and, in parallel, for sustained response to antiviral therapy. Initially response-predictive studies were focused on clinical variables, viral kinetics and HBV mutants. With the foundation of our laboratory in 2000, basic studies on HBV- and HCV-immune reactivity were initiated. From that time large-scale clinical HBV intervention studies were combined with ancillary fundamental immunological studies using an intensive translational approach. For this we focussed on the T-cell response both in the peripheral and intrahepatic compartment of patients. As following step to dissect immune tolerance in HBV infection we embarked in on the role of dendritic cells (DC) and regulatory T cells (Treg). We showed that DC *ex vivo* isolated from peripheral blood are functionally impaired and that Treg levels are increased in chronic HBV patients. Both these phenomena, which could well be the reason for the weak T cell responses in chronic hepatitis B infection, are now studied in the setting of antiviral therapy.

Liver transplantation.

Liver transplantation is on the short-term a successful treatment for end-stage liver diseases, but long-term survival and quality of life are severely impaired by complications caused by continuous immunosuppressive treatment and HCV re-infection. One part of our studies concentrate on defining parameters that identify on the one hand patients who are at risk of rejection and on the other hand patients that are tolerant to their liver graft. Prediction of the risk of immune reactivity to the graft will enable individualization, and in a subgroup of patients probably reduction, of immunosuppressive therapy. For this purpose we investigate both genetic and immune parameters. Immune parameters are studied preferentially in the graft itself. For this purpose we have introduced a liver Fine-Needle-Aspiration (FNAB) technique. Secondly, we aim to promote immunological conditions that are required for the development of tolerance against liver grafts. For this, we focus on modulation of donor- and recipient DC-migration and function and on the induction of donor-specific Treg. Thirdly, we are developing novel strategies to prevent HCV-recurrence. For this part we focus on anti-viral effects of combinations of immunosuppressive drugs and on the development of a *ex vivo* gene therapy approach based on RNA interference in which the liver transplant is transfected with anti-HCV iRNA before transplantation.

Portal Hypertension.

The pathogenesis of hepatic and portal vein thrombosis is largely unclear. We investigate on an international level the etiology and prognostic factors of these rare but life-threatening liver disorders.

Scientific achievements during the last 5 years

Dendritic cells in chronic hepatitis B infection

Ex vivo isolated DCs are functionally impaired in chronic HBV patients as compared to healthy volunteers. More specifically, myeloid DCs were impaired in antigen presentation and plasmacytoid DCs exhibited a reduced IFN-alpha production. The mechanism of DC impairment is currently under investigation, as well as the function of DC during antiviral therapy.

Regulatory T cells in chronic hepatitis B infection

We showed that chronic HBV patients exhibit increased percentages of Treg in their peripheral blood, as compared to healthy controls and patients that resolved an acute HBV infection. These Tregs were

able to suppress the HBV-specific T cell responses. Further studies will include the mechanism of suppression as well as changes in frequency and functionality of Tregs under antiviral therapy.

Immune responses in antiviral therapy of chronic hepatitis B and C infection
We used the FNAB-technique to analyse phenotype, functional status and specificity of subsets of intraheptic immune cells in HBV- and HCV-infection. The use of HLA-A2 tetrameric complexes allowed demonstrating sequestering of virus-specific CD8+ T cells in the liver, also in the setting of antiviral therapy.

Parameters identifying tolerant liver transplant recipients
We identified several genetic polymorphisms in cytokine- and co-stimulatory genes that are associated with the risk of rejection after liver transplantation. In addition we found, using the FNAB-technique, that intragraft immune parameters like enhanced granzyme- and reduced foxp3-expression are strongly associated with rejection or immunological quiescence. However, durable tolerance to liver grafts is probably dependent on active inhibition of the immune response of the recipient to donor antigen. We found that after liver transplantation, levels of reguatory T-cells in peripheral blood dropped significantly, possible as a consequence of immunosuppression.

Modulation of immunological conditions to induce tolerance to liver grafts
We found that considerable numbers of DC detach from the graft during the transplantation procedure, and demonstrated that these donor-derived DC migrate via the blood circulation into the recipient. We hypothesized that these professional antigen-presenting cells initiate the rejection process by priming allogeneic recipients T-cells. However, investigating *in vivo* matured liver DC isolated from hepatic lymph nodes, we found that liver DC are relatively weak allo-stimulators and produce high amounts of the immunoregulatory cytokine IL-10. Moreover, we demonstrated that treatment of liver transplant recipients with Intravenous Immunoglobulins (IVIg) prevented acute rejection, and that the IVIg reduce the immunogenicity of DC. Presently, it is investigated whether modulation of DC by IVIg can induce tolerance to liver grafts, and thereby open a new treatment option to reduce treatment with immunosuppressive drugs.

New therapies for Hepatitis C virus (HCV) recurrence after liver transplantation
The only current treatment for end-stage liver disease due to chronic HCV infection is liver transplantation. However, re-infection of the graft by persisting HCV causes progressive recurrence of disease and has a profound impact on survival of the patient and graft. We have developed a retroviral gene transfection that blocks HCV-replication in cultured hepatocytes by RNA interference. The aim is to effectively deliver siRNA by local gene therapy during isolated perfusion of the liver graft before transplantation. Alternatively, we found that specific combinations of immunosuppressive drugs have unexpected inhibitory effects on HCV-replication.

Portal Hypertension
With support of the European Community (5th framework) we set up a European network to construct an on-line common database and to federate sample banks. We showed the importance of several genetic defects in the etiology of vascular liver disease and constructed prognostic models for intervention. Currently we investigate the role of fibrin homeostasis in the pathogenesis and site-specificity of hepatic vein thrombosis.

Future plans: special goals and approach
Chronic viral hepatitis is the result of a complex interaction between a replicating non-cytopathic virus and a down-regulated antiviral immune response We will determine whether Treg induce tolerance to HBV through induction of HBV-tolerant-DC's from DC progenitors. Vice versa we will assess whether HBV tolerant-DC's induce Treg from naive T cells. Furthermore, we will investigate whether Treg induce HBV tolerance directly through suppression of HBV-specific $CD4^+$ and $CD8^+$ T cells. These *in vitro* studies will be combined by a study in humans assessing the effect of therapy-induced HBV reduction on both pathways of Treg-induced HBV tolerance. Finally, to reverse the inadequate immune response to the virus we will induce HBV-specific activation of DC-progenitors and functionally deplete Treg from

blood of chronic HBV patients. It is our aim to develop a therapeutic strategy through vaccination with *ex vivo* modulated DC-progenitors and *in vivo* depletion of Treg to reverse HBV tolerance and thereby resolve chronic HBV-related liver disease. Similar research is being started up now in the field of chronic HCV-infection.
In the field of liver transplantation, we will try to establish which conditions are favorable for the induction of donor antigen-specific Treg. In particular we will investigate whether transplant tolerance can be induced by *ex-vivo* manipulation of DC in the graft. In addition, the feasibility to induce donor-specific hyporesponsiveness by moduation of DC with IVIg will be explored in experimental animal studies and clinical research. To improve detection of a tolerant state, we will develop a sensitive technique to quantify the recipient T-cell response against indirectly presented donor MHC. For this purpose we will use a completely new approach, namely donor antigen presentation to recipient T-cells by recipient DC genetically transduced with donor MHC-genes. Finally, we aim to develop a gene therapy approach to prevent HCV infection of the liver graft using interfering-RNA (iRNA) to inhibit HCV replication. Conditions to achieve effective lentiviral transduction during the ex vivo perfusion of the liver graft will be determined.

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Pediatric gastro-enterology: mucosal responses to bugs and drugs

Workgroupleaders	**Department**
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Goals of research: general outline

The digestive tract is under constant attack by bacteria, viruses and other noxious agents in the lumen of the GI tract. The gastrointestinal mucosa, consisting of the epithelium and the underlying immune cells, form an important line of defense against these potential harmful agents. Mucosal damage may lead to malabsorption and diarrhea, since the gastrointestinal epithelium is essential for the digestion and uptake of nutrients and for the maintenance of the liquid balance in the human body. Since many types of GI diseases lead to mucosal damage, the overall aim of research within this theme is to understand the mechanisms responsible for damage and for subsequent regeneration of the gastrointestinal mucosa in infectious diseases, inflammatory bowel diseases and after chemotherapy. The relevance is perhaps best illustrated by the high incidence of each of the studied diseases. Furthermore, the possibilities are evaluated of intervention either to prevent mucosal damage or to stimulate the recovery of mucosal functions after an insult.

Scientific achievements during the last 5 years

Damage and repair in infectious disease

Rotavirus is the most important cause of secvere gastroenteritis in children and young animals. In vivo have shown that rotavirus infection inhibits expression of functionally important enterocyte genes resulting in apoptosis at the tips of the villi and changes in epithelial homeostasis, which most likely leads to defective absorptive cell function and as such might contribute to the pathogenesis of a rotavirus infection. We have identified several different host proteins involved in infection and induction of diarrhea. In addition, using an *in vitro* model we have identified signaling pathways involved in rotavirus infection. If we block these pathways then rotavirus infectivity is also inhibited.

Damage and repair in inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are complex inflammatory bowel diseases of unknown etiology characterized by chronic inflammation of the bowel and associated with increased risk for cancer of the affected organ. We investigate several different experimental colitis models (IL10-/-, Muc2-/-, DSS-, oxazolone- or TNBS- induced colitis) in order to find out which bacterial- or host-derived factors factors might play a role in the development, perpetuation and recovery of chronic intestinal inflammation, and determine ways to either prevent disease or induce recovery. This will help to develop new therapies for treatment of CD and UC. The most relevant are the following findings. 1). Expression of the most prominent mucin in the colon (MUC2) is deficient in IBD patients having active disease. Since this mucin is an important component of the protecting mucus layer in the colon this finding indicated that the epithelial protection is compromised in these patients during active disease. 2). This is corroborated by our recent finding that Muc2 deficient mice are much more susceptible to induction of colitis than wild-type mice under SPF conditions. 3). In IL10-/- mice under germ free conditions without any sign of colitis, Muc2 expression is significantly lower than wild types under germ free conditions, indicating that IL10 directly or indirectly influences mucin gene expression. 4). Muc2 expression specifically responds to the introduction of normal enteric bacteria *in vivo*. 5. In oxazalone colitis, an experimental colitis model for IBD, it was shown that IL-13 production is a significant pathologic factor and that CD1d restricted NK-T cells are the source of the IL-13. These data thus describe a cellular mechanism underlying an experimental colitis that may explain at least in part the pathogenesis of ulcerative colitis.

Damage and repair after chemotherapy

We set up different animal models in which we were able to characterize the epithelial responses during cytostatic drug-induced intestinal damage and subsequent regeneration. Others and we have shown that in mice and rats treated with cytostatic drugs proliferating cells in the intestinal crypt are killed, but some stem cells survive. These surviving crypt epithelial stem cells play a central role in the regeneration of the mucosa after injury. By studying rats treated with methotrexate or doxorubicin, two widely used cytostatic drugs, we have obtained a very comprehensive view of the small intestinal epithelial responses, including cell proliferation, cell death, cell migration, and shifts in numbers of the different cell types. Moreover, we were able to reveal many specific changes of the expression of cell type-specific proteins (e.g. mucins, trefoil factors, sugar-degrading enzymes and transporters, anti-microbial peptides, and fatty acid binding proteins). These changes in protein expression patterns have

important implications for the metabolism of the intestine. Furthermore, we observed that social stress aggravated the damage induced by methotrexate in these animals. In addition, we observed that the epithelium surrounding Peyer's patches, mucosal lymphoid nodules, is protected from cytostatic drug-induced damage. These observations indicate that the immune system might play an important role in cytostatic drug induced damage, which is under further investigation.

Future plans: special goals and approach

The mission of the research program is to unravel the mechanisms underlying normal function and disorders of the gastrointestinal tract in childhood and adult life by means of integrated pre-clinical and clinical research. This research aims at the development of strategies for prevention, diagnosis, and treatment of gastrointestinal diseases. Specifically, we want to focus on: 1. The mucosal damage and repair, and 2. On the cross talk between luminal agents (e.g. bacteria, viruses) and the gastrointestinal epithelium, and between the epithelium and the underlying immune cells. We will extend our (inter) national collaborations with groups that complement our work. In addition, we are setting up new technologies (proteomics, genomics) in order to achieve our goals.

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Transplantation immunology

Workgroupleaders	**Department**
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Goals of research: general outline

Donor specific cytotoxicity and unresponsiveness

Manipulation of the immunosystem is necessary for successful clinical organ transplantation. This may be achieved by prescribing immunosuppressive regimen, allowing engraftment that is traded with debilitating comorbidity associated with aspecific immunosuppression. Success may also be accomplished by tapering the immunosuppresive load allowing the emergence of immunological countermechanisms leading to non-responsiveness. In the setting of clinical organ transplantation we

study donor specific alloreactivity in an attempt to understand the immunological pathways leading to success or failure. Our specific aim is to find optimal therapeutic strategies for the individual patient.

Cytokines and chemokines in organ transplantation

Cytokines and chemokines affect proliferation, differentiation, death, and the function of cells involved in numerous physiological processes. After transplantation, specific cytokine/chemokine expression profiles may be associated with ischemia reperfusion damage and acute rejection, may identify patients who have accepted their graft, may reflect the efficacy of immunosuppression, and can trace patients at risk for chronic allograft dysfunction. However, the actions of cytokines are often pleiotropic and redundant. Antagonists against a single cytokine may not have functional consequences, as other cytokines may compensate. The latter makes it difficult to block or trigger specific steps in the cascade of immune responses after transplantation. Our aim is to elucidate the role of cytokines/chemokines in the cascade of events that lead to acute rejection, graft dysfunction, and graft acceptance after clinical transplantation under conditions of immunosuppression.

Pharmacotherapy and pharmacogenetics in organ transplantation

Most immunosuppressive drugs are critical dose drugs: they have a narrow therapeutic index. Therapeutic drug monitoring (TDM) is an important tool to optimize immunosuppressive therapy after organ transplantation. Our research focusses on the combination of the following three main topics:
Pharmacokinetics (PK): kinetics of immunosuppressive drugs and drug interactions
Pharmacodynamics (PD): how to optimize drug therapy after organ transplantation, optimal efficacy with minimal toxicity. New drugs or new drug combinations, sequential regimens.
Pharmacogenetics: how do variants in genes encoding for drug-metabolizing enzymes, drug transporters, or drug targets influence PK and PD. Pharmacogenetic research aims to predict clinically important interindividual differences.

Immune tolerance and infection after liver transplantation

The only adequate treatment for end-stage liver diseases is transplantation of a healthy donor liver. Liver transplantation has become successful partly due to the development potent immune suppressive drugs. However, complications caused by immune suppression or hepatitis virus re-infection are still major obstacles for long-term transplant success. Our current research is focussed on the role of dendritic cells in the liver and allo-suppressive regulatory T cells ($CD4^+CD25^+$) in transplant tolerance and intrahepatic monitoring of hepatitis C virus re-infection after transplantation.

Ischemia reperfusion injury

Accumulation of oxidative damage to DNA is also thought to play a major role in organismal aging by compromising cellular function, triggering cell death and limiting the proliferative capacity of regenerative tissues. Clinical observations suggest an inverse relationship between ischemia time and long-term transplant success, leading to the hypothesis that this injury leads to premature aging of organs. Our research is focussed on understanding the role of oxidative damage induced by ischemia reperfusion injury in graft dysfunction. We are especially interested in the role of aging mechanisms in graft loss. Graft survival can be extended to 2-3 months. These grafts are being lost from a thrombotic microangiopathy, leading to ischemic injury of the transplant. Thus, ischemia is now the major hurdle to overcome in order to prolong xenograft survival.

Scientific achievements during the last 5 years

Cytokines and chemokines in organ transplantation

Specific intragraft mRNA expression profiles were found in acute and chronic rejection after heart transplantation and in spontaneously resolving cellular infiltrates after liver transplantation. After organ transplantation, a constantly activated TNF-α system in combination with immunosuppressive therapy contributed to the immunosuppressive status of allograft recipients. Genetic profiles enabled us to identify patients at risk for complications after heart transplantation. Proof of redundancy in the cytokine network was shown by studies that analysed the mechanism of action of various immunosuppressive agents. Blockade of the IL-2 pathway by anti-CD25 monoclonal antibodies did not prevent allograft rejection completely. Expression levels of HIF-1α, the transcription factor that is induced in the adaptive

response to hypoxia and critical for initiating the transcriptional activation of growth factors, correlated with cold ischemia time after kidney transplantation. High mRNA expression levels of cytoprotective genes (i.e. heme oxygenase [HO]-1) and vascular endothelial growth factor at the moment of transplantation are correlated with graft function early after clinical kidney transplantation.

Pharmacotherapy and pharmacogenetics in organ transplantation

Cyclosporine co-administration reduces MPA plasma concentrations. The putative mechanism of this clinically important drug interaction is a cyclosporine-induced inhibition of the biliary excretion of MPAG, thus interfering with enterohepatic recirculation of MPA. 2.

In MMF based regimens it is possible to use reduced dose cyclosporine therapy, without causing an increased incidence in acute rejections. Adequate rejection prophylaxis can be reached with anti-IL2R monoclonal antibodies in combination with tacrolimus and MMF, in a steroid free regimen.

Patients with the CYP3A5*1 allele have a higher tacrolimus dose requirement. For cyclosporine an influence of CYP3A or MDR-1 could not be demonstrated.

Immune tolerance and infection after liver transplantation

Kupffer cells in cadaveric donor livers express co-stimulatory molecules CD80 and CD86, which are involved in antigen presentation. Liver transplant recipients caring a particular polymorphism in the gene coding for CTLA-4, the inhibitory ligand for CD80 and CD86, had lower incidence of acute rejection.

Durable tolerance to liver grafts is dependent on active inhibition of the immune response of the recipient to donor antigen. We currently investigate whether liver transplant patients develop donor-antigen specific regulatory T cells (Treg), and whether these cells can effectively suppress donor-reactive T cells.

Monitoring of specific cytokines and soluble adhesion molecules in serum and bile revealed patterns specific for acute liver graft rejection and infection. The Fine-Needle Aspiration Biopsy (FNAB) technique was developed as a save and useful method to study intrahepatic immune responses. Flowcytometric assay was developed to detect hepatitis viral antigens in FNAB samples and to study the course of HCV re-infection early after transplantation.

Ischemia reperfusion injury

A rat aorta transplantation model showed that ischemia-reperfusion injury was associated with histopathological damage, and antibody-, and complement deposition in the graft. Differential expression of intragraft cytokines was found in allografts compared to syngeneic grafts, and in self-limiting versus progressive transplant arteriosclerosis. Other alloantigen-independent factors (e.g. surgery), were also found to influence the degree of arteriosclerosis.

We have introduced gas anesthesia and perioperative support that allows us to perform extensive surgery on mice. We developed a mouse model in which 60 minutes of warm ischemia of the liver leads to non-lethal liver damage.

A rat model allows us to perform thoracotomy and clamp the left lung for 120 minutes. We analyzed lung function, lung tissue, bronchoalveolair lavage fluid and thoracic lymph nodes at several days post-reperfusion. We found pulmonary dysfunction, infiltration of (a)specific immune cells, and impaired surfactant composition.

Mouse hearts made transgenic for human complement regulatory proteins perfused with human serum enabled us to study their effect on hyperacute xenograft rejection. We showed that complement regulation at C3 level significantly prolonged graft survival, whereas downstream regulation had no effect. In the discordant guinea pig to PVG/c- rat liver transplantation model, the PVG/c- rat is unable to execute complement mediated hyperacute rejection. Using transgenic pigs, we studied whether extracorporeal liver perfusion can be used as a bridging procedure for patients with acute liver failure awaiting liver transplantation. We found a beneficial effect of transgenesis with human complement regulatory factors.

Our syngeneic rat kidney transplantation model enabled us to show that abdominal gas insufflation to create a pneumoperitoneum during laparoscopic living donor nephrectomy does not adversely affect either renal function or histomorphology of kidney grafts up to one year after transplantation. Prolonged

warm ischemia associated with laparoscopic retrieval was also shown not to affect graft function or histomorphology.

Future plans: special goals and approach

Cytokines and chemokines in organ transplantation

Studies to unravel the mechanisms by which the immune system via cytokine pathways triggers graft acceptance. The grant proposal "Transplant coronary artery disease: Immune stimulation of donor heart artery wall remodelling" is submitted to the Dutch Heart Foundation. Trails with new immunosuppressive agents will be monitored to gain insight in the mechanisms of action of these drugs, and will provide further insight in how the immune system mediates anti-donor responses after transplantation. Studies to unravel the mechanisms by which donor organ protects itself against immunologic and non-immunologic injury.

Pharmacotherapy and pharmacogenetics in organ transplantation

Abbreviated AUC or concentrations at time points other than C_{min} better reflect overall drug exposure. We aim to perform studies comparing these parameters with traditional TDM to show their true value. The influence of genetic factors on PK/PD will be studied and can then be prospectively used to aid in individual dosing of immunosuppressive drugs in order to reach target concentrations and thus optimize efficacy and avoid side effects. A crucial factor is the need for well-characterized patients who have been uniformly treated and systematically evaluated. To this end we are setting up basic requirements for the collection of genomic DNA from all our future transplant recipients. Clinical Pharmacologists are trained as consultants for this aim.

Immune tolerance and infection after liver transplantation

To investigate whether transplant tolerance can be induced by ex-vivo manipulation of DC in the graft. In particular intervening with DC maturation could prevent the induction of donor-reactive T-cells and in contrast induce regulatory T-cells that prevent the allo-response. To try to establish which conditions are favorable for the induction of donor antigen-specific Treg. We aim to develop a gene therapy approach to prevent HCV infection of the liver graft using interfering-RNA (iRNA) to inhibit HCV replication. To determine conditions to achieve effective lentiviral transduction during the ex vivo perfusion of the liver graft.

Ischemia reperfusion injury

Use of knockout mice that are defective in DNA repair pathways to determine their effect on short- and long term organ survival and function using the ischemia-reperfusion induced renal dysfunction model. Apply transcriptional profiling to understand the molecular basis of ischemia-reperfusion injury in these mice. Study differences between young and old donors Infusion of stem cells in mice exposed to renal and hepatic ischemia-reperfusion injury. Apply ischemic preconditioning in experimental models and the clinic to protect livers against ischemia-reperfusion injury. Detailed study of the inflammatory response of lungs following ischemia-reperfusion injury. Investigate novel approaches to ameliorate this response. Study the factors involved in thrombotic microangiopathy following pig to primate xenotransplantation. Use of α1,3 galactosyltransferase gene knockout pigs as donors.

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Mucosal Immunology

Workgroupleader	**Department**
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Goals of research: general outline

The incidence of lung diseases such as asthma, COPD (chronic obstructive pulmonary disease) sarcoidosis, and mesothelioma has risen dramatically over the last 50 years. In addition, pneumonia, caused by pathogens such as Streptococcus pneumoniae and influenza virus, is the most common cause of death from infectious disease in the western hemisphere. All these diseases and current therapies afflict the immune system of the lung. Currently, our efforts are directed at:
(1) Elucidating the role of dendritic cells (DCs) in directing and maintaining a chronic localized immune response to the lung, by studying the mechanisms of interaction of DCs with other inflammatory cells and mediators.
(2) Identifying the molecular mechanisms responsible for the humoral immune response that is essential for host defence against bacterial pathogens, but defective in asthma and COPD.
Deeper understanding of beneficial or detrimental pulmonary immune mechanisms can provide guidelines for rational improvement of current diagnostic and therapeutic regimens.

Scientific achievements during the last 5 years

Atopic asthma

We have systematically studied the role of DCs in the pathogenesis of atopic asthma, a disease caused by a dysregulated adaptive immune response to inhaled allergens leading to eosinophilic airway inflammation. We demonstrated in an animal model of asthma that the airways of mice with eosinophilic airway inflammation contain grossly increased amounts of myeloid DCs (Koch I), that intratracheal administration of myeloid DCs to the airways of healthy naive animals induced Th2-dependent eosinophilic airway inflammation (Koch II) and that transgene-based conditional removal of DCs from the airways of diseased animals cured all the features of allergic disease (Koch III). Therefore, we have identified this cell type as a good target for therapeutic and preventive intervention in asthma. These findings were tested in a humanized SCID model of asthma where the human immune system controls inflammation in vivo in the mouse. Here also, DCs fulfilled the postulates. In particular, we have studied the role of plasmacytoid DCs in asthma and have discovered that this cell type displays immunosuppressive properties, which could be employed for improving the therapy of asthma.

Prostaglandin D(2), which binds to the D prostanoid (DP)1 and DP2 receptor, is seen as a critical mediator of asthma causing vasodilation, bronchoconstriction, and inflammatory cell influx. We have shown that inhalation of a selective DP1 agonist suppresses the cardinal features of asthma by targeting the function of lung DCs. In mice treated with DP1 agonist or receiving DP1 agonist-treated DCs, there was an increase in regulatory T cells that suppressed inflammation. These effects of DP1

agonist on DCs were mediated by cyclic AMP-dependent protein kinase A. We furthermore show that activation of DP1 by an endogenous ligand inhibits airway inflammation as chimeric mice with selective hematopoietic loss of DP1 had strongly enhanced airway inflammation and antigen-pulsed DCs lacking DP1 were better at inducing airway T helper 2 responses in the lung. Triggering DP1 on DCs is an important mechanism to induce regulatory T cells and to control the extent of airway inflammation. This pathway could be exploited to design novel treatments for asthma.

We have also found that allergen challenge causes acute accumulation of ATP in the airways of asthmatic subjects and mice with experimentally induced asthma. All the cardinal features of asthma, including eosinophilic airway inflammation, Th2 cytokine production and bronchial hyper-reactivity, were abrogated when lung ATP levels were locally neutralized using apyrase or when mice were treated with broad-spectrum P2-receptor antagonists. Adjuvant effects of ATP were due to the recruitment and activation of lung myeloid dendritic cells that induced Th2 responses in the mediastinal nodes.

The sphingosine 1-phosphate receptor agonist FTY720 is an oral immunosuppressant that retains lymphocytes in lymph nodes and spleen, thus preventing lymphocyte migration to inflammatory sites. The accompanying lymphopenia could be a serious side effect that would preclude the use of FTY720 as an antiasthmatic drug. We have shown in a murine asthma model that local application of FTY720 via inhalation prior to or during ongoing allergen challenge suppresses Th2-dependent eosinophilic airway inflammation and bronchial hyperresponsiveness without causing lymphopenia and T cell retention in the lymph nodes. Finally, a chronic asthma mouse model has been established in the lab, enabling us to study the role of DCs, T cells and formation of inducible bronchus-associated lymphoid tissue (iBALT) in chronic asthma pathology and airway remodelling.

Allergic rhinitis
Allergic rhinitis often coexists with asthma (“ united airways”). We developed an intranasal ovalbumin-driven mouse model for allergic rhinitis, characterized by nasal eosinophilic inflammation and enhanced serum levels of OVA-IgE and Th2 cytokine production in cervical lymph node. Using this mouse model we were able to show that circulating Th2 effector cells in upper airway allergy are responsible for lower airway allergy.

Mesothelioma
Exploiting the immunostimulatory capacities of DCs holds great promise for cancer immunotherapy. We have evaluated if pulsed DCs induce protective immunity against malignant mesothelioma in a mouse model. Mice receiving tumor lysate-pulsed dendritic cells before tumor implantation demonstrated protective antitumor immunity with prolonged survival and resisted secondary tumor challenge. When given after tumor implantation in a therapeutic setting, pulsed DCs prevented mesothelioma outgrowth. Thus, we demonstrated in this murine model that immunotherapy using pulsed DCs may emerge as a powerful tool to control mesothelioma outgrowth. We are currently performing a phase I study, in a project funded by the ‘Stichting Asbestkanker’, to explore the possibility of immunotherapy using DCs as adjuvant to control local recurrence after multimodality treatment for malignant mesothelioma in human.

B and T Lymphocytes
The generation and selection of a diverse and flexible antigen receptor repertoire by B and T lymphocytes is dependent on a series of developmental cell fate decisions, including the induction and regulation of Ig and TCR gene rearrangements. These decisions are implemented by the activity of signal transduction systems and specific transcription factors and occur at specific checkpoints, controlled by antigen receptors and their immature forms.

In the Department of Immunology (1999-2007), Dr. R.W. Hendriks developed several transgenic mouse models, in which B cell development, selection or activation is defective. These models include mice expressing mutant forms of Bruton’s tyrosine kinase (Btk, which is a signalling molecule downstream of the B cell receptor) resulting in immunodeficiency, spontaneous germinal center formation or autoimmunity. Autoimminity was also found in transgenic mice that have prolonged expression of the surrogate light chain pre-B cell receptor component. In various collaborations, the role

of Btk in other signalling cascades, such as chemokine receptor, Fc-Epsilon receptor and Toll-like receptor signalling was studied in different cell types.

The research aims of the lymphocyte differentiation program also comprise the study of particular transcription factors that control the in vivo developmental program of lymphoid cells. In particular Gata3 was studied, which is important in early T cell development, as well as in directing differentiation of polarized Th2 effector cells.
Collectively, over the last years technical expertise has been acquired to study lung disease, both in patients and in animal models, using cellular immunology, cell culture, flow cytometry and sorting, image analysis, molecular biology, bioinformatics and proteomics.

Future plans: special goals and approach

A chronic asthma mouse model has been established in the lab, enabling us to study the role of DCs, T cells and iBALT formation in chronic asthma pathology and airway remodelling. We are currently also directing our efforts to the effect of respiratory viruses on the function of airway DCs and iBALT formation, and study specifically the role of DCs in pulmonary defence mechanisms. We plan to set up phase II studies to investigate the potential of tumor-pulsed autologous DCs to prevent recurrences of mesothelioma.

Our transgenic mouse models in which B cell development, selection or activation is defective will be used to study asthma, infection, immunity and autoimmune phenomena in the lung. But, as both in asthma and in COPD B cells become activated that have an aberrant repertoire (recognizing allergens and auto-antigens) we also want to study the basic principles of B cell development and B cell receptor repertoire selection.

In addition to these mouse models, we will perform patient studies to investigate the molecular mechanism of the immune reactivity in COPD. As recent evidence shows that COPD has an autoimmune component and autoantibodies have been identified in COPD patients, we aim to focus on B cell selection and activation. iBALT formation is significantly increased in patients with COPD. Therefore, we will study iBALT formation and the identification of antigen specificity of B cells present in these structures, which should help us to demonstrate their role in disease pathology. Appreciation of these autoimmune processes may ultimately enable the development of novel diagnostic, prognostic, and treatment approaches for COPD, which is an otherwise medically refractory disease.

The lung has the ability to respond quickly to some pathogens through stimulation of resident antigen-specific memory B cells. But also - after exposure to a new pathogen - the lung can generate de novo both systemic and local (mucosal) antibody responses. Thus, B cell repertoire analyses should be instructive in patients with chronic, recurrent pneumonia. Such studies are needed to provide further insight on the site of pulmonary humoral host responses to bacterial challenge and optimal vaccine regimens to minimize the burden of respiratory disease caused by pathogenic bacteria.

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10 Mantel, P. Y., Kuipers, H., Boyman, O., Rhyner, C., Ouaked, N., Ruckert, B., Karagiannidis, C., Lambrecht, B. N., **Hendriks, R. W.**, Crameri, R., Akdis, C. A., Blaser, K. and Schmidt-Weber, C. B., GATA3-driven Th2 responses inhibit TGF-beta1-induced FOXP3 expression and the formation of regulatory T cells. PLoS Biol 2007. 5: e329.

Lymphoid differentiation and immunodeficiencies

Workgroupleaders	**Department**
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Goals of research: general outline

One of the most intriguing features of the specific immune system is the generation of mature B and T lymphocytes that carry immunoglobulin (Ig) molecules and T-cell receptors (TCR), which are highly specific for antigens, even when these cells have not encountered the antigens before. The generation and selection of a diverse and flexible specificity repertoire by lymphocytes is dependent on a series of developmental cell fate decisions, including the induction and regulation of Ig and TCR gene rearrangements. These decisions are implemented by the activity of specific transcription factors and enzyme systems and occur at specific checkpoints, controlled by signaling pathways downstream of antigen receptors and their immature forms. In particular, the research aims of this program comprise:

- To study the role of lymphoid-specific transcription factors that control the in vivo developmental program of lymphoid cells.
- To investigate signal transduction routes that are crucial to stem cell self-renewal and differentiation into lymphoid cells.
- To unravel the essential steps in the induction and execution of Ig/TCR gene rearrangements in precursor-B and -T cells, e.g. using immunodeficiencies as model.
- To characterize the signal transduction pathways downstream of the antigen receptors, which are essential for survival, selection and developmental progression of lymphoid cells.
- To investigate how defects in the regulation of differentiation and proliferation steps during lymphoid development result in immunodeficiencies or lymphoid malignancies.
- To translate the obtained knowledge on normal lymphoid differentiation and gene defects into novel diagnostics and opportunities for gene therapy in patients with primary immunodeficiencies (PID).

Scientific achievements during the last 5 years

The research projects included studies on hematopoietic stem cells and lymphoid differentiation in mice and man, the precise characterization of human PID, particularly agammaglobulinemia and severe combined immunodeficiencies (SCID), and exploring opportunities for gene therapy to correct PID. Our main findings and conclusions are listed below:

Lymphoid differentiation

- The human thymus is seeded by multipotent progenitors with a much broader lineage potential than previously assumed. These cells were found to resemble hematopoietic stem cells but, by analogy with murine thymocytes, apparently lack sufficient self-renewal capacity (Weerkamp et al. Blood 2006; 107:3131-3137).
- Delta1- and Jagged1-expressing stromal cells have distinct effects on the clonogenic and differentiation capacities of human multipotent CD34(+) CD38(+) progenitor cells (Neves et al. Stem Cells 2006; 24:1328-1337).
- The human thymus is a site for B-cell development (Weerkamp et al. J Allergy Clin Immunol 2005; 115:834-840).
- T-cell development in children is a dynamic process, answering the demands of a maturing and expanding immune system (Weerkamp et al. J Allergy Clin Immunol 2005; 115:834-840).
- Wnt signals mediate proliferation and cell adhesion, but not differentiation of the immature thymic progenitor pool (Staal et al. J Immunol 2004; 172:1099-1108).
- The responsiveness of thymocytes to developmental signals by Wnt proteins are regulated by differential expression of intracellular mediators rather than by abundance of receptors or ligands (Weerkamp et al. Proc Natl Acad Sci U S A 2006; 103:3322-3326).
- By combining the TCR gene rearrangement data with gene expression data, we demonstrated that a number of key events in human T cell development occur earlier than assumed previously; therefore, it is much more similar to murine T cell development than reported before (Dik et al. J Exp Med 2005; 201:1715-1723).
- Detailed multiparameter flowcytometric studies on the composition of the precursor-B-cell compartment in human bone marrow recognized eight subsets. The relative distribution of these subsets changes during ontogeny, but is stable during the first decade of life (Noordzij et al, Pediatr Res 2002; 51:159-168).
- Based on the combined Ig gene rearrangement status and gene expression profiles of consecutive precursor B cell subsets, we identified 16 candidate genes involved in initiation and/or regulation of Ig gene rearrangements (van Zelm et al. J Immunol 2005; 175:5912-5922).
- Surrogate-light-chain silencing is not critical for the limitation of pre-B cell expansion but is for the termination of constitutive signaling (van Loo et al. Immunity 2007; 27:468-480).
- We demonstrated that naive mature B lymphocytes, but not transitional B lymphocytes, undergo in vivo homeostatic proliferation in the absence of somatic mutations in the periphery (van Zelm et al. J Exp Med 2007; 204:645-655).
- T cell-dependent B cell proliferation is substantially higher with higher frequencies of somatic hypermutation than T cell-independent responses (van Zelm et al. J Exp Med 2007; 204:645-655).

Primary immunodeficiencies

- The absence of functional BTK proteins generally leads to an almost complete arrest of human B-cell development at the pre-B-I to pre-B-II transition (Noordzij et al, Pediatr Res 2002; 51:159-168).
- Both Btk-deficient mice and BLNK/SLP-65-deficient mice manifest defects at the developmental progression of large cycling into small resting pre-B cells and have reduced Igλ light chain usage, most likely reflecting a defect in the initiation of Igλ gene rearrangements in pre-B cells (Middendorp et al. J Immunol 2002; 168:2695-2703; Kersseboom et al. J Immunol 2006; 176:4543-4552).
- Analyses of mice carrying mutant forms of Btk revealed that the main Btk autophosporylation site in the SH3 domain Y223 is not essential for in vivo Btk function, and that Btk function is partially independent of its kinase activity (Middendorp et al. J Immunol 2003; 171:5988-5996).
- The identification of a novel antibody-deficiency syndrome due to mutations in the CD19 gene (van Zelm et al. N Engl J Med 2006; 354:1901-1912).

- The absence of recombination activity in precursor-B cells of Rag-deficient SCID patients is associated with a complete B-cell differentiation arrest at the transition from pre-B-I cells to pre-B-II cells (Noordzij et al. Blood 2002; 100:2145-2152).
- Radiosensitive SCID patients with Artemis gene mutations show a complete B-cell differentiation arrest at the pre-B-cell receptor checkpoint in bone marrow (Noordzij et al. Blood 2003; 101:1446-1452).
- Defective Artemis nuclease is characterized by coding joints with microhomology in long palindromic-nucleotide stretches (van der Burg et al. Eur J Immunol 2007; 37:3522-3528).
- We demonstrated that B-cell reconstitution in a B-negative SCID patient due to an Artemis mutation required the elimination of the autologous precursor-B-cells in bone marrow, probably to create physical space in the precursor-B-cell niches. Apparently, occupation of the precursor-B-cell niches is a potential dominant factor influencing repopulation of a functional B-cell compartment in B-negative SCID (van der Burg et al. Haematologica 2006; 91:1705-1709).
- The identification of a new type of radiosensitive T-B-NK+ severe combined immunodeficiency caused by a LIG4 mutation (van der Burg et al. J Clin Invest 2006; 116:137-145).

Gene therapy
- The occurrence of leukemia in a gene therapy trial for SCID-X1 has highlighted insertional mutagenesis as an adverse effect. We demonstrated that the LMO2 oncogene is most highly transcribed in $CD34^+$ progenitor cells, which might explain the increased susceptibility of insertion into this gene.
- We demonstrated that the overexpression of LMO2 and not IL2Rgamma caused severe abnormalities in T-cell development, whereas B-cell and myeloid development remained unaffected (Pike-Overzet et al. Nature 2006; 443:E5; discussion E6-7; Pike-Overzet et al. Leukemia 2007; 21:754-763).

Future plans: special goals and approach

The current research lines will be further extended. Specifically, we will pursue our basic research on stem cells, lineage commitment and early lymphoid differentiation. We will explore the role of specific factors, which will not only provide more insight, but might also be applied for more optimal in vitro preparation of stem cells for stem cell transplantations and gene therapy.
Development of new diagnostic tools for PID patients is an ongoing process. Based on new insights, more genes will become known as candidate genes that can be affected in PID patients. We recently started to explore the mature B-cell compartment in a large series of Common Variable Immunodeficiency (CVID) patients using newly developed tools. We aim at identifying the immunological deficiencies and potentially underlying genomic defects. Once identified, the humoral deficiencies can teach us more about crucial checkpoints in lymphoid differentiation and immune responses.

Recently, we identified somatic mosaicism due to revertant mutations in several SCID patients. These patients had a seemingly leaky phenotype with some mature T lymphocytes in their peripheral blood. After sorting of these cells, DNA fingerprinting and mutation analysis, we identified revertant mutations on the mutant site or a second site. We now wish to explore the occurrence of these mutations and their effect on reverting of the deficient phenotype.

Finally, we aim to correct PID using a gene therapy approach. We aim to develop gene therapy for the most common form of T-B- SCID due to mutations in the Rag genes, and for the most common form of agammaglobulinemia due to mutations in the Btk gene. Now, T-B- SCID can be treated with bone marrow transplantation with good success if an HLA-identical donor is available. However, in case of other donors, the results are moderate to poor; in these cases gene therapy should become the alternative treatment option. Agammaglobulinemia can be treated with live-long intravenous Immunoglobulin substitution, however, due to increased susceptibility to infection, these patients develop severe lung problem already in the 3rd decade of life. Therefore, gene therapy can be regarded as a good attempt to increase quality of life in these patients.

10 most important publications

1. Dik, W.A., Pike-Overzet, K., Weerkamp, F., de Ridder, D., de Haas, E.F., Baert, M.R., van der Spek, P., Koster, E.E., Reinders, M.J., **van Dongen, J.J.M.**, Langerak, A.W., **Staal, F.J.** New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. J Exp Med 2005; 201:1715-1723. **IF 14.5**
2. van Zelm, M.C., van der Burg, M., de Ridder, D., Barendregt, B.H., de Haas, E.F., Reinders, M.J., Lankester, A.C., Revesz, T., **Staal, F.J.**, **van Dongen, J.J.M.** Ig gene rearrangement steps are initiated in early human precursor B cell subsets and correlate with specific transcription factor expression. J Immunol 2005; 175:5912-5922. **IF 6.3**
3. Weerkamp, F., Baert, M.R., Brugman, M.H., Dik, W.A., de Haas, E.F., Visser, T.P., de Groot, C.J., Wagemaker, G., **van Dongen, J.J.M.**, **Staal, F.J.** Human thymus contains multipotent progenitors with T/B lymphoid, myeloid, and erythroid lineage potential. Blood 2006; 107:3131-3137. **IF 10.4**
4. Weerkamp, F., Baert, M.R., Naber, B.A., Koster, E.E., de Haas, E.F., Atkuri, K.R., **van Dongen, J.J.M.**, Herzenberg, L.A., **Staal, F.J.** Wnt signaling in the thymus is regulated by differential expression of intracellular signaling molecules. Proc Natl Acad Sci U S A 2006; 103:3322-3326. **IF 9.6**
5. Kersseboom, R., Ta, V.B., Zijlstra, A.J., Middendorp, S., Jumaa, H., van Loo, P.F., **Hendriks, R.W.** Bruton's tyrosine kinase and SLP-65 regulate pre-B cell differentiation and the induction of Ig light chain gene rearrangement. J Immunol 2006; 176:4543-4552. **IF 6.3**
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8. Pike-Overzet, K., de Ridder, D., Weerkamp, F., Baert, M.R., Verstegen, M.M., Brugman, M.H., Howe, S.J., Reinders, M.J., Thrasher, A.J., Wagemaker, G., **van Dongen, J.J.M.**, **Staal, F.J.** Gene therapy: is IL2RG oncogenic in T-cell development? Nature 2006; 443:E5; discussion E6-7. **IF 26.7**
9. van Loo, P.F., Dingjan, G.M., Maas, A., **Hendriks, R.W.** Surrogate-light-chain silencing is not critical for the limitation of pre-B cell expansion but is for the termination of constitutive signaling. Immunity 2007; 27:468-480. **IF 18.3**
10. van Zelm, M.C., Szczepanski, T., van der Burg, M., **van Dongen, J.J.M.** Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. J Exp Med 2007; 204:645-655. **IF 14.5**

Transplantation and genetic modification of hematopoietic stem cells & immune reconstitution after transplantation

Workgroupleader	**Department**
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Dr. J.W. Gratama	Oncology/Medical Tumor-immunology

Goals of research: general outline

Within this theme there is a longstanding research effort in murine models for human diseases and nonhuman primate models for stem cell biology and transplantation, which is concerned with the manipulation of immune modulation and the development of gene transfer for therapeutic purposes. Hematopoietic stem cell transplantation (SCT) is currently an important therapeutic modality for many malignant hematological disorders, and it's use for the treatment of metastatic solid tumors is under investigation as well as its development for gene transfer as a therapeutic modality. Transplant-related morbidity and mortality of allogeneic SCT is still significant due to acute and chronic graft-versus-host disease (GVHD) and opportunistic infections (mainly reactivations of endogenous herpes viruses). Our research focusses on:

The identification and treatment of patients with an impaired immune recovery after transplantation at high risk for specific progressive viral infections (JWG, JC).
The development of interventions, including cytokine intervention therapy, to improve immune recovery after transplantation (JC, EB).
The development of alternative approaches to facilitate engraftment, including the selective inductionof donor allo-antigen-specific tolerance by use of tolerogenic dendritic cells (EB, JC).
The development of gene therapeutic approaches for inherited diseases, spin-off acquired diseases, further development of hematopoietic and mesenchymal stem cell transplantation using genemarked cells (GW).

Scientific achievements during the last 5 years

Identification of transplant (SCT) recipients at high risk for viral infections.
In the context of effective prevention of cytomegalovirus (CMV) disease in SCT recipients using pre-emptive treatment with ganciclovir, quantitative viral load monitoring and with monitoring of CMV specific T-cells identifies patients at high risk for CMV related complications.
The development of a highly sensitive real time quantitative PCR assay for Epstein Barr Virus (EBV) allowed us to study the behavior of the virus in SCT recipients and to develop an effective pre-emptive strategy using the anti-B-cell monoclonal antibody rituximab. When combining the results of frequent viral load monitoring with those of a tetramer-based study of EBV specific CD8+ T-cell recovery, the positive predictive value for LPD improved from 40% (based on viral load monitoring only) to 100% based on virology and immunology.

Development of new therapeutic approaches to improve immune recovery after SCT.
B- and T-lymphocyte function after transplant may be deficient for a prolonged period of time following SCT. In a murine transplantation model across syngeneic or allogeneic MHC-matched and MHC-mismatched barriers (T-cell and B-cell deficient RAG-1-/- mice receive RAG-1+/+ bone marrow), thymic function was assessed by a real- time quantitative PCR assay of T-cell receptor rearrangement excision circles (TRECs). We found that the frequency of TRECs depends on age, genetic background, and input of bone marrow derived lymphocyte precursor cells. Furthermore, the administration of Interleukin-7 (IL-7) posttransplant resulted in enhanced recovery of T-cells, both after a T-cell depleted and T-cell replete BMT.

Tolerogenic dendritic cells to promote engraftment.
We have optimized conditions for the in vitro generation of murine dendritic cells (DC) from hematopoietic progenitor cells in bone marrow and characterized these DC extensively.

Employ fluorescent marking of human hemopoietic stem cells to study stem cell biology and develop gene transfer methodology.
The working group early on began to employ fluorescent marking of human hemopoietic stem cells to study stem cell biology and develop gene transfer methodology. This has facilitated the identification of the growth factors relevant for proliferation and maintenance of hemopoietic stem cells and the development of develop high efficiency retrovirus mediated gene transfer methods of long-term repopulating stem cells. An effective methodology has been developed to identify signal transduction pathways involved in primary stem cell development and proliferation in mouse, nonhuman primate and human stem cells. Lentiviral vectors have more recently been added to the arsenal experimental high efficiency gene transfer.

Future plans: special goals and approach

Stem cell research as well as gene therapy development is envisaged to offer a great potential for the development of therapeutic modalities for inherited as well as acquired diseases within as well as outside the hemopoietic system. Considering that translation of the basics into clinical therapy requires a large, multidisciplinary research effort and extensive preclinical evaluation, we concentrate on:
For CMV and EBV, as for any antigen, current coverage of the total immune response by tetramer technology is far from complete. As an alternative, we are studying the use of protein-spanning peptide pools in short-term T-cell stimulation assays (read out: cytokine production). The advantage of this approach is that any functional protein-specific T-cell can potentially be detected, and thus has the

potential of a universally applicable diagnostic tool. We are now prospectively studying the effectiveness of this approach in SCT recipients. At the same time, the identification of CMV carrying patients (and healthy donors) with functional CMV protein specific T-cells will allow the analysis of the fine specificities of these responses by testing the T-cells against smaller peptide pools and finally against individual peptides.
We will evaluate in our murine model whether the combination of IL-7 and other cytokines, including stem cell factor (SCF) and Flt-3 ligand allows for better T-cell recovery and whether that effect is exerted at the level of lymphocyte precursor output from the bone marrow, at the thymus-level, or through post-thymic peripheral expansion. Functional restoration will be assessed by protection against murine viral CMV infection and rejection ability of third party skin grafts, and various T-cell parameters in-vitro. The combined knockout of RAG-2 and the common-gamma-chain has resulted in a viable, but severely immunosuppressed, murine model, that allows for engraftment of human stem cells. By backcrossing to a Balb/c background, we will use these mice for functional analysis of immune competence directed against the murine CMV virus. Clinically, it is anticipated that early phase I-II clinical trials with IL-7 can be designed within 2 years upon completion of our pre-clinical studies and the non-human primate studies.
Dendritic cells have a dual role, they play a central role in the initiation of antigen-specific immune responses but they are also pivotal for the induction and maintenance of peripheral tolerance. The major challenge for us will be to induce a shift in the delicate balance between immunostimulatory and tolerogenic DC in vivo. We will pursue this by administration of DC exposed in vitro to certain cytokine (combinations) or by the in vivo administration of cytokine (combinations) that either mobilize the protolerogenic properties of precursor DC or directly modify the functions of DC in vivo. A second challenge is the translation of our preclinical results into clinical application. We aim to develop a DC-based alloantigen-specific tolerization strategy for allogeneic stem cell transplantation in the clinic.
The research focusses on development of gene therapeutic approaches for inherited diseases, spin-off acquired diseases, development of hematopoietic and mesenchymal stem cell transplantation using gene marked cells, analysis of signal transduction pathways (HOXB4, wnt, STAT5) involved in hemopoietic stem cell proliferation and repopulation, e.g., by systematic identification of target genes of signalling pathways. This should provide insight into the molecular basis of stem cell proliferation and maintenance as well as generate tools to enable a (transient) selective advantage to gene-modified stem cells rather than the loss of repopulating capacity that has significantly slowed down clinical gene therapy development. Emphasis will be placed on retroviral/lentiviral insertional mutagenesis in the context of cell type specific gene expression, likely the major risk of gene therapy, for which the group has developed mouse models.

10 most important publications (selected from 65 SCI publications)

1. **Gratama JW**, Van Esser JWJ, Lamers CHJ, Tournay C, **Löwenberg B**, Bolhuis RLH, **Cornelissen JJ**. Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8+ T lymphocytes in T-cell-depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection. Blood 2001;98:1358-1364. **IF 9.6**
2. Meij P, Van Esser JWJ, **Niesters HGM**, Van Baarle D, Miedema F, Blake N, Rickinson AB, Leiner I, Pamer E, **Löwenberg B**, **Cornelissen JJ**, **Gratama JW**. Impaired recovery of Epstein-Barr virus (EBV)-specific CD8+ T lymphocytes after partially T-depleted allogeneic stem cell transplantation may identify patients at very high risk for progressive EBV reactivation and lymphoproliferative disease. Blood 2003;101:4290-4297. **IF 9.6**
3. Broers AEC, **Meijerink JPP**, **van Dongen JJM**, van Sluijs S, **Löwenberg B, Braakman E**, **Cornelissen JJ**. Quantification of newly developed T cells in mice by real-time quantitative PCR of T cell receptor rearrangement excisional circles. Exp Hematol 2002;30:745-750. **IF 3.4**
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5. Commeren DL, Van Soest PL, Karimi K, **Löwenberg B, Cornelissen JJ**, **Braakman E**. Paradoxical effects of interleukin-10 on the maturation of murine myeloid dendritic cells. Immunol. 2003; in press. **IF 2.7**
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7. **Braakman E**, Steijart MMC, **Zijlmans JMJM**, Slaper-Cortenbach I, Boogaerts MA, **Löwenberg B**, Verdonck LF. A randomized multicenter comparison of CD34+ selected progenitor cells from blood versus from bone marrow in recipients of HLA-identical allogeneic transplants for haematological malignancies. Exp Haematol 2003; in press. **IF 3.4**
8. Neelis KJ, Visser TP, Dimjati W, Thomas GR, Fielder PJ, Bloedow D, Eaton DL, **Wagemaker G**. A single dose of thrombopoietin early after myelosuppressive total body irradiation prevents pancytopenia by promoting short-term multilineage spleen repopulating cells at the transient expense of bone marrow repopulating cells. Blood 92: 1586-1597, 1998. **IF 9.6**
9. Van Hennik PB, Verstegen MMA, Bierhuizen MFA, Limón A, Wognum AW, Cancelas JA, Barquinero J, **Ploemacher RE**, **Wagemaker G**. Rapid Publication: Highly efficient transduction of the green fluorescent protein gene in human umbilical

cord blood stem cells capable of cobblestone formation in long-term cultures and multilineage engraftment of immunodeficient mice. Blood. 1998 Dec 1;92(11):4013-22. **IF 9.6**

10. Wognum AW, Visser TP, Peters K, Bierhuizen MF, **Wagemaker G**. Stimulation of mouse bone marrow cells with kit ligand, flt3 ligand, and thrombopoietin leads to efficient retrovirus-mediated gene transfer to stem cells, whereas interleukin 3 and interleukin 11 reduce transduction of short- and long-term repopulating cells. Hum Gene Ther. 2000 Oct;11(15):2129-2141. **IF 5.1**
11. Verstegen MMA, Wognum AW, **Wagemaker G**. Thrombopoietin is a major limiting factor for selective outgrowth of human umbilical cord blood cells in non-obese diabetic/severe combined immunodeficient recipient mice. British Journal of Hematology, 2003, 122, 837–846 **IF 3.1**

Immune regulation and autoimmunity

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Prof. dr. J.D. Laman	Immunology
Dr. R.Q. Hintzen	Neurology
Dr. B.C. Jacobs	Neurology
Prof. dr. E.P. Prens	Dermatology
Dr. E. Lubberts	Rheumatology

Goals of research: general outline

Chronic inflammation and autoimmune disease are leading causes of morbidity, psychosiocal burden and economic loss in Western society. In view of the central role of the innate and adaptive immune system in these diseases, detailed insight into immune regulation is a requirement for rational development of diagnosis and (immuno) therapy. The research school Molecular Medicine has an extensive and active programme in immune regulation and autoimmunity, consisting of a close collaboration between clinical and pre-clinical departments. Specifically, the departments of Neurology (Prof.Dr. P.A. van Doorn, Dr. R.Q. Hintzen, Dr. B.C. Jacobs), Dermatology (Prof.Dr. H.A.M. Neumann, Prof.Dr. E.P. Prens) and Rheumatology (Prof.Dr. J.M.W. Hazes, Dr. R.J.E.M. Dolhain, Dr. E. Lubberts) have structurally integrated their immunological research groups within the Dept. of Immunology. There is additional close collaboration on the role of microbial compounds in immune regulation with the Depts. Virology (Prof.Dr. A.D.M.E. Osterhaus), Medical Microbiology & Infectious Diseases (Prof.Dr. H.A. Verbrugh, Dr. H. Endtz, Prof.Dr. A. van Belkum, Dr. J. Hays). Furthermore, there is close collaboration with departments of Internal Medicine (Prof.Dr. T.J. Visser) and Epidemiology (Prof.Dr. A. Hofman, Prof.Dr. M.M.B. Breteler) on thyroid autoimmune disease, and with the department of Psychiatry on immune regulation in major affective disorders (Prof.Dr. M. Hengeveld, Drs. V. Bergink, Drs. N. van Beveren). The contribution of immune response gene polymorphisms to inflammation and autoimmune diseases is investigated in collaboration with the Dept. Pediatrics.

Selected diseases of interest are type I diabetes, thyroiditis, major affective disorders and schizophrenia (all having a major immunoneuroendocrine component), rheumatoid arthritis (RA) and the related disorder Sjögren syndrome, psoriasis, and the demyelinating diseases multiple sclerosis (MS) and Guillain-Barré syndrome (GBS). The general premise is that the immunological mechanisms driving the multifactorial pathophysiology in the different diseases of interest are highly analogous. These mechanisms include influences of the neuroendocrine system, genetic polymorphisms of molecules involved in cellular interaction, and microbial infection, as well as the leukocyte effector functions mediating tissue damage. Close collaboration with clinical researchers who are well trained in immunology allows joint elaboration of scientifically relevant research questions, construction of well-characterized patient cohorts, and evaluation of experimental (immuno) therapy. Combining researchers with different backgrounds (e.g. molecular biology, cellular immunology) working on these different diseases in a single integrated team significantly stimulates scientific discussion and output. The joint expertise allows coverage of a broad area of approaches and technology, ranging from patient cohort studies via functional in vitro and genetic analyses of patient material to several animal disease models in rodents and non-human primates. Some departments have initiated new research programs on autoimmunity recently, while others have a longstanding international track record (e.g. Dept.

Neurology). The MS research is organized in the MS Centre ErasMS (head Dr. R.Q. Hintzen), supported by a programme grant. The Biomedical Primate Research Centre (Dr. B.A. 't Hart and colleagues, Rijswijk) participates in this MS Centre. Research topics in this subtheme include basic immunopathogenic mechanisms (e.g. molecular mimicry in GBS and MS); immune-endocrine interactions (e.g. diabetes, thyroiditis, amelioration of MS, thyroiditis and RA during pregnancy); molecular signaling pathways in chronic inflammation (transcription factors in psoriasis); experimental immunotherapy (e.g. antibodies against costimulatory molecules and cytokines); immune regulation by external factors (e.g. UV irradiation and skin inflammation, infection and MS activity); immune function and disease activity in MS; immune regulation by aberrant development and activity of antigen presenting cells (e.g. diabetes, histiocytosis).

Scientific achievements during the last 5 years

Endocrine autoimmunity

It was discovered that dendritic cells, the antigen-presenting cells, are functionally defective and in a pro-inflammatory state in the two spontaneous animal models of endocrine autoimmune disease, the NOD mouse and BB-DP rat. Correction of this abnormality led to a prevention of the disease in the animal models (Nicolic, thesis; Geutskens, thesis; and Tse, thesis).

It was discovered that bipolar disorder patients suffer from autoimmune diseases 3x more frequent then the general population and that there is a shared genetic vulnerability factor for endocrine autoimmune disease and major mood disorders (Knijff, thesis; Hillegers, thesis).

It was discovered that the shared genetic vulnerability factor for bipolar disorder and endocrine autoimmune disease is a pro-inflammatory state of monocytes, macrophages and dendritic cells giving support to the "macrophage theory of depression" (Vonk et al., Hillegers thesis).

The abnormal pro-inflammatory state of monocytes of bipolar patients and endocrine autoimmune patients was catched in a specific "mRNA signature" of pro-inflammatory genes. This signature occurred prior to the development of bipolar disorder and most likely can be used as a blood test for (the susceptibility) for major mood disorders and endocrine autoimmune disease.

Local precursors for dendritic cells in the pancreas

*S*tudies on the origin of the dendritic cells, which accumulate in the pre-diabetic pancreas of NOD mice, revealed the presence of local proliferating precursors for dendritic cells in the initiation phase just before the accumulation of dendritic cells starts. In addition precursors for dendritic cells and macrophages are already detected in the embryonic pancreas indicative for a role of these locally generated cells in the normal development of the pancreas.

Moncytes and dendritic cells in Sjögren's syndrome

The role of monocytes and dendritic cells in the pathogenesis of Sjögren's syndrome was studied in the peripheral blood of patients and in the NOD mouse model for Sjögren's syndrome. In dendritic cells isolated from salivary glands of NOD mice an abnormal localization of the chemokine receptor CCR5 was found. In addition an abnormal degradation of fractalkine (a chemokine attracting a subset of monocytes) was detected in infiltrated salivary glands. Monocytes obtained from Sjögren's syndrome patients showed an increased expression of genes that were induced by interferon–alpha, indicative for an increased systemic interferon-alpha activity. Study of monocyte subsets in Sjögren's syndrome revealed an increased presence of the CD16+ subset.

These data support the hypothesis, that abnormalties in this target organ for autoimmunity as well as abnormalities in monocytes and monocyte-derived cells play a role in the development of the disease.

Central nervous system inflammation and MS

Inflammation plays a role in most neurodegenerative diseases. The sub-unit Central Nervous System (CNS) Inflammation of the Erasmus MC originates from the clinical and scientific focus on multiple sclerosis (MS). MS is the most common cause of neurological disability in young people in the European community. Many inflammatory CNS disorders can mimick MS, such as viral infections (meningo-encefalitis), systemic autoimmune diseases (SLE, Sjögren syndrome), sarcoidosis and neurobehçet.

Despite distinct pathologies in the clinical array of these CNS disorders, several common pathways appear to exist. In MS it is probably a myelin directed T- and B-cell mediated autoimmune process that

stands at the base of the pathology. MS is caused by a fatal interaction of yet poorly identified genes (e.g. HLA) with environmental factors (viral infections, specifically EBV, vitamin D and perhaps smoking).

Aim of this program is to enhance insight in the different routes that lead to white matter inflammation as well as neuronal and axonal damage, mainly using MS as a model.
The unit has a strong focus on biology and translational medicine, with a general theme around 'Biological determinants of the disease course'. Intense collaborations exist within the following areas: Clinical Neurology, Immunology, Genetics, Epidemiology, Virology, MRI and Proteomics. Clinical material consists of data and samples from various cohorts as well as DNA of Dutch multiplex MS families and a unique family with 26 persons suffering from MS, which is the largest MS pedigree in the world. In addition, post mortem tissue of brain and lymph node of MS patients is readily available.
Examples of subthemes in the research program are:

- Proteomic analysis of cerebrospinal fluid, using a large sample bank linked with clinical data.
- Development of novel neuroprotective therapy.
- The triggering role of EBV ('Pfeiffer') virus in CNS demyelination, using serology, PCR and
- immunohistochemistry.
- MS in children.
- Prognostic markers after a first attack of possible MS.
- MS gene identification in Dutch MS families (microarrays) and SNP analyses.
- Immunological and endrocrinological effects of pregnancy on MS.
- T-cell surveillance of latent herpes simplex infection.
- Clinical characteristics of neurobehçet
- The role of innate immune signals and antigen presenting cells in autoimmune demyelination.

Pathogenesis of the Guillain-Barré syndrome
The Guillain-Barré syndrome (GBS) is the most common form of acute neuromuscular paresis. Patients with GBS have a rapidly progressive immune-mediated neuropathy resulting in severe paresis of limb and respiratory muscles, from which patients may die. Research at Erasmus MC showed that GBS is a molecular mimicry mediated disease in which preceding infections trigger the production of toxic cross-reactive antibodies to neural structures. In about 40% of patients these antibodies are directed to neural glycolipids or gangliosides. *Campylobacter jejuni* is the predominant cause of infection in GBS and lipo-oligosaccharides from these bacteria indeed exactly mimic gangliosides. Infusion with donor immunoglubulins is an effective treatment in GBS, although the mechanism of action of this treatment is unknown.
Four important issues remain unsolved in GBS:

- *What are the immuno-targets in patients without anti-ganglioside antibodies?* Pilot studies have identified new targets, but these need to be tested in the available large cohorts of patients, in relation to neurological deficits and prognosis.
- *What is the cellular mechanism driving the production of these cross-reactive antibodies?* Our recent studies indicate that *C. jejuni* directly activates dendritic cells and B-cells. This *in vitro* model for GBS will therefore enable us to determine the responsible cellular pathways.
- *Can genetic host factors explain why only 1 in 1000 persons with a Campylobacter infection develops GBS?* We are studying single nucleotide polymorphisms in immune response genes, which may determine this abnormal response to infection.
- *What mechanism of action is responsible for the therapeutic effect of immunoglobulins?* Several serological and cellular models have been developed to identify the effective fractions of these immunoglobulins and clarify the mechanisms of action.

At the Erasmus MC there is a unique collaboration between the departments of Neurology, Immunology, Medical Microbiology & Infectious Diseases regarding GBS research. Central to this collaboration is the patient-related laboratory research, which gives us excellent opportunity to address these four study objectives and in which students are gladly invited to participate.

Achievements in the last 5 years

GBS

Regarding the identification of new immuno-targets we found that some GBS patients produce antibodies to structures formed by mixtures or complexes of gangliosides. These structures are also present on Campylobacter, indicating that the antibodies to these complexes are also induced by cross-reaction.

The cellular mechanism driving the onset of GBS was further substantiated. The oligosaccharide structure of Campylobacter influences dendritic and B-cell activation.

A new gene polymorphism was identified associated with GBS. This polymorphism influences the levels of mannose binding lectin. As predicted persons with a gene polymorphism inducing high levels of this complement activator had more severe GBS.

A clinical model was developed to predict the prognosis of immunoglobulin treated patients with GBS. Based on this model and initial serological experiments patients that may profit form a second course of this treatment can be identified.

Rheumatoid Arthritis

Different arthritis mouse models have been established including the collagen-induced arthritis (CIA) model and the antigen-induced (flare) arthritis (AIA) model. In these models we have shown that IL-23 induces Th17 polarization in splenic T cells from type II collagen-immunized DBA-1 mice and is critical for Th17 specific IL-22 expression (EULAR, Barcelona 2007 and ACR, Boston, 2007 both oral presentation, manuscript in preparation). In addition, IL-23 is critical in the progression of joint inflammation and bone erosion in a non-autoimmune model of arthritis (manuscript in preparation). Moreover, T cell specific over expression of GATA3 protects against severe joint inflammation during experimental arthritis by suppression of Th17 polarization (manuscript in preparation). Our ongoing work demonstrates high IL-17 and IL-23 levels in the synovial fluid of patients with active RA and circulating human Th17 cells were noted in early RA patients in contrast to healthy controls. RA patient cohorts (REACH, Rotterdam Early Arthritis Cohort and the PARA, Pregnancy Amelioration of Rheumatoid Arthritis) are being extended. Using material from the REACH it was shown that 1,25-dihydroxyvitamin D3 modulates dexamethasone effects on IL-4 and IL-17 production by CD4+ lymphocytes in rheumatoid arthritis patients (manuscript in preparation). Furthermore, using material from the PARA, it was shown that disease activity and prednisone use influence birth weight in Rheumatoid arthritis pregnancies and the first data from our prospective nationwide cohort study (PARA) were presented showing whether rheumatoid arthritis improves during pregnancy.

Psoriasis

An ex vivo human skin model was established and is in use for functional analysis of immune regulation, and especially inflammation in human skin The involvement of type I interferons in the chronic phase of psoriasis was demonstrated. Further studies revealed that the innate receptors involved in Interferon signaling are upregulated in vivo in psoriasis, indicating that this pathway is also potentially involved in the initation of psoriasis. A mouse model representing a psoriasis-like dermatitis was developed. This model represents a powerful tool to investigate candidate drugs for psoriasis.

Patents and spin outs

The research programme has spawned three spin off life science companies, SkinTec BV (treatment of dermatitis using protease inhibitors), Biotempt BV (peptide immunomodulators), and Biotempt Anti-sepsis BV (peptide immunomodulators for treatment of sepsis). These companies are based on a large series of patent applications. The exploitation of scientific advances is aimed to generate additional funding for our research activities.

Future plans: special goals and approach

The monocyte genes previously identified to be aberrantly expressed in bipolar disorder, thyroid autoimmune disease and type 1 diabetes will be further developed to be used in clinically applicable assays to distinguish various subforms of type 1 diabetes (e.g.childhood versus adult forms, including LADA) and autoimmune thyroiditis (e.g. the post partum form) and to make a prognosis possible of major mood disorders. Novel immunosuppressants (PDE4B inhibitors, biologicals and COX-2 inhibitors) will be investigated for their therapeutic potential in the treatment of major mood disorders.

To identify genes that determine cause and course of MS. To search for markers in cerebrospinal fluid that are associated with clinical relevant forms of the disease. To unravel immunological mechanisms that are associated with disease modulation during infection and during pregnancy. Furthermore in depth analysis will follow on the interplay between dendritic cells, vitamin D, Tregs and IL-17/IL-23 pathways. Animal models and postmortem MS tissue are being employed for assessment of the role of costimulatory molecules and functional consequences of antigen routing from the CNS to draining lymphoid organs.
In the future the four most important unsolved issues in GBS research described above will be approached. To find new immunotargets genetically engineered Campylobacters will be used to synthesize glycoarrays. The mechanism of antibody production will be studied in several human cellular model systems. The role of genetic host factors will be studied in a world wide initiative to collect cohorts of patients and develop new screening techniques. New forms of treatment have been developed that will be tested in multicenter trials.
The function and regulation of type I IFN's in dendritic cell subtypes and in keratinocytes from patients with psoriasis and healthy controls will be further assessed. Genetic profiling will be performed in patients undergoing UV-therapy for psoriasis. In parallel projects in human and experimental animal models, functional and molecular effects of UVB irradiaton on skin immune regulation will be assessed. The psoriasis-like dermatitis model in mice will be analyzed in depth and will be further developed for psoriasis target-drug screening. A 3D skin equivalent model will be developed for functional experiments with the aim to dissect the pathomechanism of psoriasis in terms of key pathogenic cells and key molecular pathways.
The research of the Department of Rheumatology is focused on early diagnosis of RA and to understand the immunological mechanism(s) critical in the development of chronic destructive arthritis (RA). The longstanding PARA and REACH cohorts will be continued and extended. In particular, the role of IL-23/Th17 immune pathway and Vitamin D in the development of chronic and destructive arthritis and other inflammatory diseases will be examined. Arthritis mouse models will be continued and specific Th17 related cytokine / cytokine receptor knockout or transgenic mice will be used. In addition, the interaction between hormones and immune processes will be investigated in the PARA-study. Osteo-immunological studies will be set up including inflammatory osteoarthritis and aging. Pathogenic function of auto-antibodies (anti-CCP, RF) will be elucidated using mouse genetic knockout models and humanized arthritis models.

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