

Programma van de Nederlandse Vereniging voor Parasitologie op 8 Maart 1995 te Lunteren.

12.00 uur - 13.30 uur Voorjaarsvergadering

programma van de voordrachten

13.30 uur - 13.45 uur **Polderman e.a.**

De serodiagnostiek van *Strongyloides*-infecties

13.45 uur - 14.00 uur **Thomas e.a.**

P. falciparum apical membrane antigen-1 (83/AMA-1) is a target antigen for inhibitory immune responses

14.00 uur - 14.15 uur **Slierendrech e.a.**

P. falciparum liver stage immunisation by irradiated sporozoite inoculation in chimpanzees

14.15 uur - 14.30 uur **Kocken e.a.**

Expression of the human malaria parasite *Plasmodium vivax* candidate PV66/AMA-1 in the methylotrophic yeast *Pichia pastoris*

14.30 uur - 14.45 uur **Hartskeerl e.a.**

Genetic and immunological characterization of the microsporidian *Septata intestinalis* and detection of microsporidia in clinical specimen by PCR

14.45 uur - 15.00 uur **Buijs e.a.**

Effect of treatment with anti-IL5 or anti-IL4 on the immuno-inflammatory reaction in the lungs of *Toxocara canis*-infected mice

15.00 uur - 15.15 uur **Schallig e.a.**

Sheep immune responses to *Haemonchus contortus* and the biochemical analysis of parasite antigens

15.15 uur - 15.30 uur theepauze

15.30 uur - 15.45 uur **Oskam e.a.**

De diagnose van viscerale leishmaniasis: het nut van de directe agglutinatie tests

15.45 uur - 16.00 uur **Homan e.a.**

Development of a PCR for the detection of *Neospora*

16.00 uur - 16.15 uur **Beetsma e.a.**

Induction of transmission blockade in mice infected with *Plasmodium berghei*

16.15 uur - 16.30 uur **Lensen e.a.**

Activated human neutrophils in a mosquito bloodmeal reduce transmission of *Plasmodium falciparum*

16.30 uur - 16.45 uur **Hermsen e.a.**

CD4 and CD8 positive T-lymphocytes independently effect development of *P. berghei* cerebral malaria

16.45 uur - 17.00 uur **Pinelli e.a.**

Lysis of *Leishmania infantum* infected macrophages by cytotoxic T-cell lines generated from asymptomatic infected dogs

17.00 uur - 17.15 uur **Tielens.** Succinate production and the function of complex II of the respiratory chain during the life cycle of *Schistosoma mansoni*

The *P.falciparum* vaccine candidate molecule PF83/AMA-1 is highly immunogenic in naturally occurring human infections, and as a full length baculovirus expressed recombinant molecule.

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We have developed a capture ELISA, using baculovirus expressed full length *P.falciparum* PF83 apical membrane antigen-1, to assess specific immune response. Recombinant material captured to ELISA plates with a mAb directed against the C-terminal (intra-cytoplasmic) domain of PF83/AMA-1 has been used to analyze endemic, human antibody responses after naturally occurring infection, and rat and mouse responses following immunisation with recPF83/AMA-1. Human responses were initially characterised at the total IgG level in a number of populations, including a highly characterised Senegalese cohort. Compared with responses to total mature schizont parasite material that were also measured in our assay, and to responses reported for other malarial antigens, total IgG responses to PF83/AMA-1 developed to surprisingly high levels (end point titres of 1: 30,000 and above were not uncommon) and developed early in life in highly endemic areas. Recombinant material used to immunise rats and mice for functional analyses was also found to be highly immunogenic. A variety of adjuvants were able to induce strong, parasite inhibitory IgG responses (assessed in vitro) using immunising regimes that used as little as 1ug per dose, delivered over three doses. Thus, functionally important immune responses to this vaccine candidate antigen were induced in a relatively adjuvant independent fashion, and immunising doses required to achieve this effect were relatively low. This is important in the light of the need to produce conformationally intact material for immunisation, and suggests that even a low efficiency recombinant expression system producing correctly folded material may be of value in the path to human vaccine trials with this molecule.

Family history of *Giardia lamblia*: resistance to nitro-imidazol and sensitivity to Albendazole

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Two out of three children from a healthy family had a well-documented infection with *Giardia lamblia*, not responding to repeated treatment with nitro-imidazols. Infection cleared after treatment with albendazole. The other child and father had untreated asymptomatic *Giardia* infection and did not respond to albendazole but to metronidazole.

Methods: of pre-treatment faecal samples cysts were purified and excystated. The strains were named: Nij2-Nijm5.

In vitro testing for drug resistance was performed by adding antibiotics (metronidazole, albendazole) in increasing concentrations to the culture medium.

Iso-enzyme patterns and DNA-fingerprinting was done of strains and clones.

Results:

Growth ceased in medium with Albendazol $\geq 0.1 \mu\text{g}/\text{ml}$.

After repeated testing clone 10, Nij2, Nij3, 4 and 5 were all resistant to metronidazole, up to concentrations of $80 \mu\text{g}/\text{ml}$. Resistance is not only a matter of selection.

After storage in liquid nitrogen the strains were no longer resistant; resistance seems not to be a stable property.

Iso-enzyme and DNA comparisation showed Nij2 to be from a different group than Nij3-5. This means that two or three different *Giardia* strains were circulating within one family.

Development of a PCR for the detection of *Neospora*

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Neospora is a recently recognized cyst-forming coccidium frequently causing fetal abortion and neonatal mortality in cattle and severe neurological disease in dogs. Little is known about the human infectivity of this parasite that is morphologically almost identical to *Toxoplasma*. To obtain more insight in the occurrence of *Neospora* in hosts and environment a *Neospora* specific PCR was developed. A PCR based on the amplification of the ITS1 region of ribosomal DNA originally developed for the characterization of *Toxoplasma* strains produced PCR products of identical molecular weight for *Toxoplasma* and *Neospora caninum*. Sequence analysis revealed several differences in the ITS1 region between *Toxoplasma* and *Neospora*. Isolates of *Toxoplasma* (both virulent and non- mice virulent) on the other hand did not reveal any polymorphisms, indicating that this region is highly conserved in *Toxoplasma*. *Toxoplasma* and *Neospora* could be distinguished by restriction analysis of the PCR products from both parasites. Based on the sequence differences detected between the two species a *Neospora* specific PCR was produced that detected both *N. bovis* and *N. caninum*.

Sheep immune responses to *Haemonchus contortus* and the biochemical analysis of parasite antigens.
H. Schallig, M. van Leeuwen, W. Hendrikx, B. Verstrepen, W. Bernadina, W. van der Aar, F. Kooyman,
J. Boersema, J. Poot, M. Eysker & A. Cornelissen.
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The nematode *Haemonchus contortus* is a major pathogen, primarily affecting the abomasum of ruminants. The present study focused on the immune responses of non-immune and *H. contortus*-immune sheep against excretory/secretory (E/S) antigens.

Characterization of the antibody responses against E/S material with ELISA demonstrated that IgG1 was the dominant antibody isotype in serum. Immunoblotting showed that three groups of antigens were strongly recognised by immune sera: i.e. molecules with MW's having around 50-55 kD, 24 kD and 15 kD, respectively. Lymphocyte proliferation assay experiments showed a significant increased response of PBMC's obtained from immune sheep against the E/S material. The cellular response is at present analyzed in more detail.

Biochemical analysis revealed that the 50-55 kD antigens possessed proteolytic activity, but the function(s) of the lower MW antigens is at present unknown. We have determined the N-terminal amino acid sequence of these proteins. Corresponding oligonucleotides were used in PCR to amplify cDNA from *H. contortus*. A CDNA library was screened with the PCR products as probes, the genes were isolated and are at present sequenced.

De diagnose van viscerale leishmaniasis: het nut van de directe agglutinatie test.

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De goudstandaard voor de diagnose van viscerale leishmaniasis is het aantonen van *Leishmania* amastigoten in punctaten. Deze invasieve manier van diagnose is echter bewerkelijk en betrekkelijk ongevoelig. Daarom zijn er een aantal serodiagnostische technieken ontwikkeld, waaronder de directe agglutinatie test (DAT). Het principe van de DAT berust op de agglutinatie van gekleurde *Leishmania* promastigoten door de anti-*Leishmania* antistoffen in het serum. Door een verdunningsreeks van het serummonster te maken kan de titer bepaald worden.

De DAT is snel en eenvoudig en daarom geschikt voor gebruik in de tropen. Een nadeel is dat het antigeen bij 4°C bewaard moet worden. Daarom hebben we een DAT, gebaseerd op een gevriesdroogd antigeen, ontwikkeld. Deze DAT is binnenkort als commerciële kit verkrijgbaar. Een vergelijking met de oorspronkelijke DAT liet zien dat met het gevriesdroogde antigeen dezelfde gevoeligheid (92%) en specificiteit (99,7%) te bereiken waren.

Klinische verschijnselen kunnen aan de seroconversie vooraf gaan. Bij een tweede DAT na 2-4 weken bleek veelal dat de titer duidelijk positief geworden was.

Gedurende het eerste half jaar na behandeling bleef de titer gelijk, waarna deze geleidelijk afnam. Het duurde veelal minstens een jaar alvorens de titer negatief werd.

Genetic and immunological characterization of the microsporidian *Septata intestinalis* by PCR

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Septata intestinalis has recently been recognized as a new microsporidian in HIV-infected persons. Transmission electron microscopy revealed that *S. intestinalis* had many morphological characters in common with *Encephalitozoon*. However, due to a number of distinct ultrastructural differences *S. intestinalis* has been classified as a species of a new genus.

We have further examined phenotypic and genetic aspects of *S. intestinalis*. Phenotypic characterization was performed by analysis of the protein composition and antigenic structure of spores from various microsporidian species by SDS-PAGE and Western blotting. The genetic characterization consisted of the determination of the sequence of the *S. intestinalis rrs* gene encoding the small subunit ribosomal RNA (srRNA), restriction fragment length polymorphism (RFLP) analysis of amplified *rrs* genes and establishment of the degree of sequence identity between *rrs* genes of various microsporidian species.

Based on the results from this study it is concluded that *S. intestinalis* is so closely related to *E. cuniculi*, the type species of *Encephalitozoon*, that it should be reclassified as *Encephalitozoon intestinalis*.

P. falciparum liver stage immunisation by irradiated sporozoite inoculation in chimpanzees

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Irradiation of *P. falciparum* sporozoites renders them nonviable, but leaves them able to induce protective responses in humans. The abortive liver stages of such attenuated parasites may play an important role in the induction of protection. To gain insight into the liver stage specific immune responses that take place after inoculation with irradiated sporozoites, experiments in the chimpanzee model (the only host other than man reproducibly susceptible to sporozoite infection) have been initiated. Two animals have been inoculated with irradiated sporozoites. Cellular and humoral responses were assessed from peripheral blood lymphocytes (PBL), and locally infiltrating lymphocytes from liver biopsies. Lymphoproliferative and cytotoxic responses against sporozoite and liver stage specific antigens were measured. Responses against stage-specific antigens were evident in an animal which received 180 Gy irradiated sporozoites, while a higher dose (300 Gy) treated animal showed a more restricted reaction pattern. Cytotoxic assays with PBL showed positive responses in the high dose treated animal against a pool of liver stage peptides. Liver derived lymphocytes from the low dose treated animal showed responses to peptides from CS and liver stage specific antigens. The animals have recently been challenged with infectious sporozoites. Analysis of protection, as assessed by blood stage infection is currently being performed.

P. falciparum apical membrane antigen-1 (PF83/AMA-1) is a target for inhibitory immune responses.

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The AMA-1 molecular family has given promising vaccine results in rodent and simian parasites. The family is surprisingly well conserved between the malaria species so far observed. However, in contrast to the 66 kDa molecule reported for all other species, *P.falciparum* has an 83kDa molecule. The difference in molecular weight is accounted for by an N-terminal region unique to *P.falciparum*, downstream of which the molecule is highly similar to that found in the simian and rodent parasite species. This difference has raised questions as to whether the promise of AMA-1 in other vaccine models can be fulfilled for *P.falciparum*. We have expressed the full length, transmembrane, PF83/AMA-1 molecule in a baculovirus expression system (chosen for the perceived need to reproduce native folding and di-sulphide bonding). Purified material has been used to immunise rats and mice, and immune sera from these animals has effectively inhibited merozoite reinvasion of *P.falciparum* in vitro. Monoclonal IgG's, prepared with cell lines derived from immunised rats, also inhibit parasite invasion, and bind to conformational, reduction sensitive, epitopes on native parasite and baculovirus derived material. This is in agreement with data derived from other parasite species, and is evidence that PF83/AMA-1 should be considered as a promising vaccine candidate antigen for *P.falciparum*.

Expression of the human malaria parasite *Plasmodium vivax* vaccine candidate PV66/AMA-1 in the methylotrophic yeast *Pichia pastoris*.

C.H.M. Kocken, M.A. Dubbeld and A.W. Thomas.

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Plasmodium vivax is one of the four *Plasmodium* species causing malaria in humans. Although not giving high mortality rates like *P. falciparum*, high morbidity rates are a burden to the people living in endemic areas. The asexual blood stages of malaria parasites are responsible for the clinical manifestation of malaria and a vaccine against these stages would prevent the symptoms of malaria infection. An important asexual blood stage vaccine candidate is the Apical Membrane Antigen (AMA-1) family of proteins, a family of well-conserved transmembrane proteins of *Plasmodium* species expressed at the late schizont stage of development. Antibodies directed against this protein are potent blockers of merozoite invasion of erythrocytes.

We have cloned the gene fragment encoding the extracellular region of AMA-1 from *P. vivax* into a *P. pastoris* shuttle vector and prepared recombinant *P. pastoris*. Upon induction with methanol recombinant PV66/AMA-1 is secreted into the culture medium at a level of 700 mg/L. Due to the presence of three N-glycosylation sites the protein is hyperglycosylated, which was not expected to occur in the *P. pastoris* expression system. Glycosyl groups can be removed from the protein using N-glycosidase F, and glycosylation can be prevented using unusual high concentrations of tunicamycin. Rats have been immunised with the recombinant PV66/AMA-1 and monoclonal antibodies have been prepared. These are now being tested for reactivity with *P. vivax* schizonts and for biological activity.

De sero-diagnostiek van *Strongyloides*-infecties

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Universiteit van Suriname, Paramaribo.

Serologisch onderzoek naar infectie met *Strongyloides stercoralis* bleef geruime tijd onbevredigend. Regelmatig werden patiënten gezien bij wie rhabditiforme larven bij faecesonderzoek konden worden aangetoond terwijl de serologische respons zó gering was dat de test-uitslag als negatief werd beoordeeld. Anderzijds konden bij veel van de serologisch positieve patiënten bij zorgvuldig faecesonderzoek geen larven worden gevonden.

Twee jaar ervaring met een ELISA op basis van *S. ratti* antigeen leert dat

- * de test moeilijk goed te standaardiseren is,
- * de test zeer gevoelig is bij patiënten bij wie op klinische of epidemiologische gronden sprake is van herhaalde auto-infectie (of hyperinfectie),
- * de test een geringe gevoelighed heeft bij patiënten die kort geleden geïnfecteerd werden en bij wie (nog) geen aanwijzingen voor autoinfectie bestaan.

De voorlopige conclusies zijn gebaseerd op de vergelijking van resultaten van serologisch en parasitologisch onderzoek bij drie groepen patiënten:

- * ex-gedetineerden die werkzaam waren in de Japanse krijgsgevangenkampen in Birma,
- * asymptomatische bewoners in Paramaribo,
- * patiënten uit de praktijk van klinisch-diagnostisch onderzoek van het Laboratorium voor Parasitologie te Leiden.

De bevindingen bij onderzoek van deze groepen patiënten worden besproken.

Behandeling met anti-IL-5 en anti-IL-4 van muizen geïnfecteerd met *Toxocara canis*

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Toxocara canis geïnfecteerde BALB/c muizen werden behandeld met anti-IL-5 of anti-IL-4 producerende rat hybridoma cellen om respectievelijk de toenamen van eosinofilen en IgE te remmen. Het effect op het verloop van door migrerende *T. canis* larven veroorzaakte ontstekingen in de longen werd bestudeerd. Anti-IL-5 toegediend twee dagen voor and 5 dagen na infectie remden volledig de toename van eosinofilen in bloed en bronchoalveolair lavaat (BAL). De significant toegenomen albumine concentratie in het BAL van geïnfecteerde muizen werd niet beïnvloed door toediening van anti-IL-5 of het Ig isotype (contrôle), suggererend dat de aan eosinofilen toegedachte rol bij permeabiliteitsveranderingen van longcapillairen marginaal was. Longcoupes van niet-geïnfecteerde en van met anti-IL-5 behandelde geïnfecteerde muizen, specifiek gekleurd om IL-5 and IFN- γ positieve cellen aan te tonen, waren negatief voor deze cellen, terwijl geïnfecteerde en met isotype of fys. zout behandelde muizen IL-5 positieve cellen bevatten. Opvallend was de toename van IFN- γ positieve cellen bij geïnfecteerde en anti-IL-5 of isotype behandelde muizen. Klaarblijkelijk stimuleert rat eiwit in *T. canis*-geïnfecteerde muizen de toename van IFN- γ positieve cellen. Toediening van anti-IL-4 rat hybridoma cellen op dag 0 en 7 p.i. had geen invloed op aantal en typen cellen in het BAL. Anti-IL-4 remde de IgE toename in geïnfecteerde muizen met ongeveer 70% in serum en BAL op dag 14, maar niet meer op dag 28 p.i.. Isotype behandeling stimuleerde de IgE productie. Bij langdurige aanwezigheid van larven en dus van抗原 blijkt een type B_{ϵ} cel te worden gegenereerd die IgE produceert onafhankelijk van IL-4. Het gebruik van "non-self" eiwitten om specifieke cytokinen te neutraliseren blijkt onverwachte en ongewenste neveneffecten te veroorzaken.

Darmprotozoa en diarree in de huisartspraktijk

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Doel: Optimalisering van de diagnostiek naar protozoaire verwekkers van diarree in de huisartspraktijk, waarbij onderzoek verricht wordt naar een verbetering van laboratoriumtechnieken van parasitologisch faecesonderzoek in combinatie met een analyse van de klinische relevantie en een kosten-batenanalyse van een dergelijke verfijning.

Plaats: Stichting Artsenlaboratorium Haarlem.

Opzet: Explorerend, descriptief.

Methode: In 50 geselecteerde huisartspraktijken in het verzorgingsgebied van het Artsenlaboratorium wordt bij alle patiënten die zich aanmelden met intermitterende diarreelachten of diarree die langer aanhoudt dan 10 dagen parasitologisch faecesonderzoek verricht. Bij het laboratoriumonderzoek (blind uitgevoerd) wordt gebruik gemaakt van een voor Nederland nieuwe procedure (SAF-gefixeerd faecesmateriaal, IJzerhaematoxyline-Kinyoun kleuring) waarbij, ter vergelijking, ook de algemeen geaccepteerde procedure (verse faeces, Ridley-concentratie) gevuld wordt.

Preliminaire resultaten:

1-Bij onderzoek van met IJzerhaematoxyline gekleurde preparaten van SAF-gefixeerde faeces worden bijna 2 maal zoveel potentieel pathogene protozoa gevonden dan bij Ridley-onderzoek van verse faeces.

2-Veel van de meeropbrengst is te danken aan het efficiënt opsporen van *Dientamoeba fragilis*. Er worden echter ook meer gevallen met *Giardia lamblia* en *Entamoeba histolytica* gediagnosticserd.

3-De operationele aspecten (inter-/intrabeoordelaars variatie, objectivering meerwerk) van het onderzoek van SAF-gefixeerde faeces in combinatie met gekleurde preparaten is onderwerp van verdere studie.

4-De klinische relevantie van de meeropbrengst aan potentieel pathogene protozoaire infecties behoeft verdere evaluatie en is eveneens onderwerp van studie.

A. Lensen, M. Bolmer, G.J.van Gemert, W.Roeffen,
W.Eling and R.Sauerwein

ACTIVATED HUMAN NEUTROPHILS IN A MOSQUITO BLOODMEAL REDUCE TRANSMISSION OF *PLASMODIUM FALCIPARUM*

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Next to (parasitized) red blood cells, white blood cells form a major component of a mosquito bloodmeal. A bloodmeal may contain $\pm 10\text{-}50.10^3$ WBC; the vast majority is composed of neutrophils which may efficiently phagocytose and kill developing gametes despite the mosquito midgut environment and non physiological temperature around 25 °C. Phagocytosis induces a respiratory burst, which can be studied *in vitro* by a luminol-dependent chemiluminescence assay. Phagocytosis is influenced by complement, specific antibodies and cytokines. The presence of complement has a strong synergistic effect on the luminescence response in combination with antibodies recognizing gamete surface proteins and cytokines as TNF, IFN and IL-6. Furthermore sera from endemic areas have been identified demonstrating an increased luminescence response. Testing these sera in transmission experiments reveals a transmission reduction in the presence of viable neutrophils, whereas in tests without white blood cells no reduction is observed. In the "conventional" transmission-blocking assay no viable white blood cells are included. Therefore the measurement reflects only the direct effect of serum factors on the sporogony. The results suggest a cell-mediated transmission reduction, enhanced by the presence of antibodies, complement and cytokines, that might contribute to the total transmission reduction.

Lysis of *Leishmania infantum* infected macrophages by cytotoxic T cell lines generated from asymptomatic infected dogs

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Evidence on the role of cytotoxic T lymphocytes as one of the effector mechanisms in protection against protozoan parasites has recently been accumulated. Although, cytokines such as interferon-gamma (IFN- γ), produced by the Th1 subset has been shown to play a major role against *Leishmania* infection, evidence for other protozoan parasites such as *T. gondi* and *Plasmodium* spp. shows that the protective capability of T-cells may also be due to their cytolytic activity. We have generated *Leishmania*-specific T-cell lines from peripheral blood of experimentally infected asymptomatic dogs, that express cytolytic activity against *Leishmania infantum* infected target cells. Specific lysis by this T-cell lines could be induced after stimulation with irradiated autologous peripheral blood mononuclear cells as antigen presenting cells and parasite soluble antigen. The cytotoxic effector cells generated, lysed infected autologous, but not allogeneic target cells, indicating that the cytotoxicity measured in this system is MHC-restricted. We also observed that these T-cell lines produce IFN- γ following antigen stimulation. These results suggests that in addition to the protective role of cytokines such as IFN- γ , cytotoxic T lymphocytes may also play an important role in protection against this parasitic infection.

CD4 and CD8 positive T-lymphocytes independently effect development of *P. berghei* cerebral malaria.

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C57Black mice infected with *Plasmodium berghei* (K173 or Anka strain) develop cerebral malaria (CM). The role of T-cell subsets in the development of CM in our mouse model was analysed by *in-vivo* depletion of CD4+ and CD8+ T-cells in intact or thymectomized mice. For the experiments both K173 and Anka infected mice were used. Results did not show a parasite strain dependent difference in the protection to CM. In the untreated control group 11% of the mice did not developed CM. When mice were depleted for CD4+ or CD8+ T-cells resp. 76% and 91% were protected against cerebral malaria. Depletion of CD4+ or CD8+ T-cells could at least be performed until approximately 0.5 to 1 day before untreated controls normally die of cerebral malaria and still protect mice against CM.

The results indicate that development of cerebral malaria depends on the expression of T-cell dependent immunopathologic reactions and that both CD4+ and CD8+ T-cells play a role in the development of CM in our *P. berghei*-mouse model.

Abstract voor lezing op woensdag 8 maart 1995,
Voorjaarsvergadering van de Ned. Ver. Parasitol.

Succinate production and the function of complex II of the respiratory chain during the life cycle of *Schistosoma mansoni*.

A.G.M. Tielens, Laboratory of Veterinary Biochemistry, Utrecht University

Adult schistosomes, which live in the blood vessels of mammals, have a fermentative metabolism and produce mainly lactate. The free-living stages of *S. mansoni* (cercariae and miracidia), on the other hand, have an aerobic energy metabolism. They degrade their endogenous glycogen reserves mainly to carbon dioxide. When cercariae penetrate the final host, they transform into schistosomula. During this biological transformation with its many morphological and physiological changes, a biochemical transition also occurs. Schistosomula were found to switch very rapidly from Krebs cycle activity to the production of lactate. This rapid transition is not caused by a lack of oxygen, but by the mere presence of external glucose combined with the specific kinetic properties of schistosomal hexokinase.

During the life cycle of schistosomes another, comparable transition from an aerobic towards a more fermentative metabolism occurs when free-living miracidia develop into sporocysts in the intermediate host, a snail. Again, even in the presence of oxygen, lactate becomes the major end product of glucose breakdown. Sporocysts were found to be facultative anaerobes; they adjust their metabolism to the variable conditions inside the snail. In the presence of oxygen they are mainly dependent on Krebs cycle activity but also produce lactate, whereas under the anaerobic conditions that sometimes occur in the snail, they switch towards lactate and succinate production.

It was shown that this succinate is largely produced via phosphoenolpyruvate carboxykinase (PEPCK). This pathway, which is known to occur in most other parasitic helminths, had never been demonstrated in schistosomes before. The formation of succinate via this pathway implies that complex II of the respiratory chain of schistosomes can function not only as a succinate dehydrogenase (SDH), but also as a fumarate reductase (FRD). In general, SDH interacts with ubiquinones in the respiratory chain, whereas FRD interacts with quinones with a lower standard redox potential, like rhoquinone. It was demonstrated that in schistosomes during their life cycle there is a relationship between the nature of their metabolism, the quinone composition and the functioning of complex II.

Apparently, the genetic information for the synthesis of the two different quinones and the two types of complex II is present, but is only expressed in the stage inside the intermediate host and not in the comparative stage in the final host like all other parasitic helminths do.

Posters:

Developmental changes in structure and function of complex II of the respiratory chain in parasitic worms.

J.J. van Hellemond, M.H. Roos and A.G.M. Tielens

Membrane biogenesis and lipid metabolism in *Schistosoma mansoni*.

J.F.H.M. Brouwers and A.G.M. Tielens

Family history of *Giardia lamblia*: resistance to nitro-imidazol and sensitivity to AlbendazoleKortbeek, L.M.¹, Homan W.¹, Knapen, F. van¹, Tolboom, J.J.M.²¹ Laboratory of Parasitology and Mycology; National Institute of Public Health and Environmental Hygiene Bilthoven,² Pediatrics , University Hospital Nijmegen

Two out of three children from a healthy family had a well-documented infection with *Giardia lamblia*, not responding to repeated treatment with nitro-imidazoles. Infection cleared after treatment with albendazole. The other child and father had untreated asymptomatic *Giardia* infection and did not respond to albendazole but to metronidazole.

Methods: Of pre-treatment faecal samples cysts were purified and excystated. The strains were named: Nij2-Nijm5.

In vitro testing for drug resistance was performed by adding antibiotics (metronidazole, albendazole) in increasing concentrations to the culture medium.

Iso-enzyme patterns and DNA-fingerprinting was done of strains and clones.

Results:

Growth ceased in medium with Albendazole $\geq 0.1 \mu\text{g/ml}$.

After repeated testing clone 10, Nij2, Nij3, 4 and 5 were all resistant to metronidazole, up to concentrations of $80 \mu\text{g/ml}$. Resistance is not only a matter of selection.

After storage in liquid nitrogen the strains were no longer resistant; resistance seems not to be a stable property.

Iso-enzyme and DNA comparisation showed Nij2 to be from a different group than Nij3-5. This means that two or three different *Giardia* strains were circulating within one family.