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Geachte collega de Geus,

Hierbij zend ik U een aantal abstracts van lezingen en posters, gepresenteerd tijdens onze najaarsvergadering op 17 november te Amsterdam.

Zoudt U zo vriendelijk willen zijn deze te plaatsen onder de kop:

NETHERLANDS SOCIETY FOR PARASITOLOGY

Autumn meeting Amsterdam, 17 November 1989

De volgorde van de abstracts is rechtsboven aangegeven.

Mag ik U tevens vragen of de abstracts van onze vergaderingen in 1988 (voorjaarsvergadering MS 4428 en najaarsvergadering MS4556) reeds zijn gepubliceerd? In onze bibliotheek beschikken wij niet over het T.G.M.. Mochten ze al in druk zijn verschenen mag ik U dan vriendelijk verzoeken om mij een fotokopie van de betreffende pagina's te sturen? Bij voorbaat mijn hartelijke dank.

Met vriendelijke groet,



Fred Borgsteede, secretaris N.V.P.

1

SCHISTOSOMES: IMMUNOMODULATION IN THE SNAIL-INTERMEDIATE HOST
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Immunomodulation is one of possible immuno-evasive strategies in schistosome-snail interactions.

In studying the compatible relationship between the avian schistosome *Trichobilharzia ocellata* and its snail intermediate host *Lymnaea stagnalis*, the influence of infection -during successive stages of parasite development (0,2,4,6,8,10 weeks post exposure)- on the circulating haemocytes was investigated. In a study on gross morphological and phenotypic (surface epitopes recognized by lectins and by monoclonal antibodies [1] as parameters), as well as functional aspects (proliferative, phagocytic and endogenous peroxidatic activities), a general activation of the system was found throughout the period studied, except at 6 weeks post exposure [2].

Further analysis of morphological aspects by means of morphometry showed that in uninfected snails cells recognized by 5 monoclonal antibodies detecting surface epitopes all spread less than other cells recognized by 5 cytoplasm-markers. This clear distinction lasts throughout the period studied and is lost under the influence of infection. Moreover, flow-cytometry revealed the distribution of cell size within the haemocyte population to be similar in all 40 snails studied, whereas the sizes of subpopulations recognized by either of 3 surface markers differ among themselves. These features are irrespective of snail age, infection, or developmental stage of the parasite.

Functional studies on changes in phagocytic activity, studied in vitro, showed a shift from effects mediated by altered humoral factors, early in the infection, to cell-bound changes, later during the infection [3].

In studying specific anti-trematode activity, by means of chemotaxis, cells of juvenile *L. stagnalis* appeared to be attracted more readily by secretory/excretory products of *T. ocellata* than by those of *Schistosoma mansoni*.

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2

GLYCOGEN METABOLISM BY *SCHISTOSOMA MANSONI* IN VIVO

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A permanent canula was installed in hamsters infected with *S. mansoni* to study the incorporation of glucose from the bloodstream of the host into the glycogen reserves of the parasite, while the latter remained undisturbed inside the veins of this host. Tracer amounts of [U-¹⁴C]glucose and [1-³H]glucose were injected consecutively, to analyse possible changes with time in the glycogen synthesis of individual worm pairs [1]. The results showed that all individual worm pairs continuously synthesized glycogen, and at a fairly constant rate. Only small differences in glycogen synthesis could be observed between parasites isolated from different locations in the veins of the hamster.

Therefore, *S. mansoni* worm pairs do *not* have alternating periods of glycogen degradation and synthesis, and degradation and synthesis do *not* occur at different sites in the bloodstream of the host.

Replenishment of the endogenous glycogen reserves was shown not to be induced by a marked decrease of the glycogen levels, but occurred continuously and slowly. At the estimated rate it will take *S. mansoni* worm pairs about 24 hours to replace their entire endogenous glycogen reserves.

Reference

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IN TRYPANOSOMES mRNAs ARE COMPOSED OF CLASS II AND III TRANSCRIPTS

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To dissect the transcription process in *Trypanosoma brucei*, we have studied the trypanosomal RNA polymerases (1). On the basis of an analysis of the genes encoding the largest subunits of trypanosomal RNA polymerases, we could demonstrate that *T.brucei* contain genes for the largest subunit of RNA polymerase I, II and III (1), as well as a second RNA polymerase II gene, which is confined to the African trypanosomes, all exhibiting antigenic variation (1, 2).

To determine which RNA polymerase-class is involved in the transcription of the trypanosome-specific genes encoding the surface antigen (VSG) and mini-exon-derived RNA (mini-exon genes), we have performed nuclear run-on experiments under conditions, in which the three RNA polymerase classes can be differentially activated (3). The outcome of our experiments suggests that the VSG genes of *T.brucei* are transcribed by an alpha-amanitin resistant RNA polymerase II. Moreover, our data also suggest that the mini-exon genes, which encode the 5'end of each trypanosomal mRNA, are probably transcribed by RNA polymerase III.

Chromatographical purification of the RNA polymerase II fraction from trypanosomal extracts indicated that only a single alpha-amanitin sensitive RNA polymerase II is present in *T.brucei* (3). This suggests that RNA polymerase II is most likely modulated by an additional factor. Studies to isolate this putative factor are in progress.

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THE ROLE OF HELMINTH INFECTIONS IN THE MASS MORTALITY OF HARBOUR SEALS (*PHOCA VITULINA* L.) IN NORTH-WEST EUROPE IN 1988

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Osterhaus et al.(1) demonstrated that a phocine distemper virus caused the mass mortality of harbour seals (*Phoca vitulina* L.) in the North-East Atlantic in 1988. From the end of May onwards dead seals were found along the Dutch coast. Several seals were deep frozen for further examinations. Ninety four of these seals became available for parasitological investigations. Lungs, heart and gastrointestinal tract were macroscopically and microscopically investigated. The following parasites and infection percentages were found: Nematoda : *Dipetalonema spirocauda* (24.5 %), *Otostrongylus circumlitus* (6.4 %), *Parafilaroides gymmurus* (24.5 %), *Ascaridoidea* spec. (58.5 %). Trematoda : *Phagicola septentrionalis* (66.0 %), *Cryptocotyle lingua* (74.5 %), Cestoda : *Diphyllobothrium* spec. (8.5 %), Acanthocephala : *Corynosoma strumosum* (70.2 %). The presence of worm species was not evenly distributed over the age classes. Seals less than one year old harboured less parasites. The highest percentages were found in seals which were 2 - 3 years old. The number of worms per seal varied greatly. The highest burden of Ascarids was 253, of *P. septentrionalis* 123,000, of *C. lingua* 112,000 and of *C. strumosum* 251. A comparison of the present results with those described in the Dutch literature shows that the same species were present and that worm numbers were not higher than before the 1988 mass mortality. It is therefore concluded that helminth parasites did not cause the mass mortality.

References

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(2)

PROSPECTS FOR SEPARATION OF *LYMNAEA STAGNALIS* HAEMOCYTE SUBPOPULATIONS

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Monoclonal antibody- and lectin binding studies indicate the presence of haemocyte subpopulations in the pond snail *Lymnaea stagnalis*, intermediate host of the avian schistosome *Trichobilharzia ocellata*.

(1). Study of these subpopulations may offer important information on haemocytes in their role as the major effector in the defence reactions of the host against the invading parasite. In other invertebrates successful isolation of functionally different haemocyte subpopulations has been described (cf. 2). In general, it is difficult to manipulate and separate invertebrate blood cells due to aggregation of haemocytes.

An "anti-clumping" (anti-c) buffer (30 mM NaCl, 20 mM glucose, 6 mM tri-sodium citrate, 5 mM citric acid, 2 mM EDTA; 120 mOsm, pH 4.5 - 7.8), yielding monocellular suspensions, was introduced to overcome this problem. *L. stagnalis* haemocytes suspended in anti-c remain viable for at least 48h; when placed back in a physiological environment, these haemocytes display normal behaviour (attachment to glass and plastic, cell spreading, phagocytosis).

Currently haemocyte subpopulation isolation trials, using preformed continuous Percoll density gradients -- ranging from 36% to 21% Percoll -- are in progress. Preliminary results indicate that isolation of distinct haemocyte subpopulations is feasible.

After isolation of haemocyte subpopulations the cells will be studied for properties determining schistosome/snail immunological compatibility. The techniques applied offer valuable tools and information for culture of *L. stagnalis* haemocytes in the future.

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(6)

PRIMARY AND REACTIVATED TOXOPLASMA INFECTIONS IN CARDIAC TRANSPLANT
RECIPIENTS STUDIED WITH IMMUNOBLOTTING

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Toxoplasma gondii may constitute a cause of life-threatening disease after cardiac transplantation. At highest risk for developing disease are the toxoplasma seronegative recipients of the heart of a seropositive donor. Approximately 50% of them may develop primary infection [1]. Data on reactivation of latent infection or of reinfection in seropositive recipients are scarce. SDS-polyacrylamide gelelectrophoresis and immunoblotting was used to analyze the IgG and IgM antibody response during primary and reactivated infection. Primary infections detected by seroconversion in ELISA's for IgG and IgM were distinguished in immunoblots from chronic infections by the appearance of anti-35 kD antibodies in the IgG and anti-6 kD antibodies in the IgM blot. Intensified staining of already existing faint bands did occur. One seronegative recipient of the heart of a seropositive donor ~~that~~ WHO developed toxoplasmosis converted to seronegativity in ELISA's 1.5 year after seroconversion, but a distinct 35 kD IgG band remained visible in immunoblot. In seropositive recipients changes in antibody level measured by ELISA's were reflected in changes in immunoblot. Reactivation of latent infection was observed as either increased staining of specific and natural antibody bands and/or the appearance of one or more bands. In IgG blots the appearance was noted of 6 kD, 20 kD, 25 kD and a variety of bands of higher molecular weight. In IgM blots bands appeared from 27 kD to approximately 105 kD.

Reference

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DETECTION OF PNEUMOCYSTIS INFECTIONS BY INDIRECT IMMUNOFLUORESCENT
ASSAY AND TRADITIONAL STAINING.

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Experience and skill is involved in obtaining a correct diagnosis of *Pneumocystis pneumoniae* in reviewing lung-derived preparations stained with silvermethenamine stain for cyst walls and Giemsa stain for the detection of trophozoites and sporozoites of *Pneumocystis*. The results of traditional stained preparations were compared with those obtained with three different monoclonal antibodies as primary antibody in indirect immunofluorescent assay. Commercially available monoclonal antibodies were used: 3F6 (courtesy Linder, SBL, Stockholm, Sweden; presently available from Dakopatts A/S, Denmark), MerifluorTM *Pneumocystis* (kit from Meridian Diagnostics Inc., USA) and DetectIF *Pneumocystis carinii* (kit from Northumbria Biologicals Ltd., UK). The IgM-FTTC conjugates used were goat F(ab')₂ anti mouse (TAGO, USA) in assay with the 3F6 monoclonal, and conjugates provided as kit component. Broncho-alveolar lavage fluid samples and 1 sputum obtained from patients admitted to the University Hospital Dijkzigt, Rotterdam, suspected for *pneumocystis pneumoniae*, were used. Most patients were HIV seropositive. All samples (55) obtained during one year in sufficient quantities for repeated testing were studied. Slide preparations were read immediately and studied independently and blindly by three investigators. When in the Merifluor assay processing of specimens was modified by ethanol fixation/inactivation instead of paraformaldehyde and heat-fixation by flame and the introduction of enzyme digestion, results by all three assays were similar. With IF an increased sensitivity of 7% (4 samples) was obtained. In all four cases parasites were found only sporadically. Morphological characteristics of parasitic stages are to be considered in interpretation, still demanding experience and skill in reading of slides with less than abundant parasites. At present the IF assay with 3F6 monoclonal is used in case parasites are not found with traditional stained preparations of lavage material from HIV seropositive patients.

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IS 55 kD PROTEIN OF HUMAN HEPATOCYTE PLASMA MEMBRANE ESSENTIAL FOR
RECOGNITION OF SPOROZOITE INVASION AND DEVELOPMENT OF HEPATIC STAGES
OF *PLASMODIUM FALCIPARUM*?

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After a bite of an infected mosquito, the injected sporozoites specifically invade hepatocytes and transform into exoerythrocytic parasites (EE). The EE of *P. falciparum* can only develop in man, primates and in primary cultures of human hepatocytes (HH). HepG2-A16 cells (Hep), a cloned line of well-differentiated human hepatoblastoma cells are highly susceptible for invasion of *P. berghei* sporozoites and for the development of EE. Although these cells are also susceptible for *P. falciparum*, development of EE has never been observed in these cells. To determine whether differences exist in the mechanism of sporozoite invasion between HH and Hep cells, the influence of a 55 kD protein of HH plasma membranes on the sporozoite invasion was investigated. This 55 kD protein is a putative receptor of sporozoite invasion as proposed in a separate study. The invasion of sporozoites was blocked by this protein in HH as well in Hep cells. However, rabbit anti-55 kD protein antiserum had no effect on the invasion in Hep cells, while invasion was significantly inhibited in HH. In addition, no proteins reactive to the antiserum were detected in Hep cells (Western blot), while a single, 55 kD protein band was demonstrated in HH. These findings indicate that *P. falciparum* sporozoites employ different mechanisms for invasion into HH and into Hep cells. Furthermore, this 55 kD protein may be essential for sporozoite invasion which leads to the development of EE parasites.

RECOGNITION OF HUMAN PLASMA MEMBRANE PROTEINS BY *PLASMODIUM FALCIPARUM* SPOROZOITES: PUTATIVE HEPATOCYTE RECEPTORS FOR SPOROZOITE INVASION.

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After a bite of an infected mosquito and injection of sporozoites, the sporozoites invade specifically and rapidly hepatocytes. The mechanisms underlying hepatocyte invasion and the host specificity are as yet not fully understood. To determine whether surface proteins of hepatocytes may be involved in the sporozoite invasion, plasma membrane proteins were prepared from human livers with CHAPS and labelled with ^{125}I (IODOGEN). The labelled proteins were incubated with *P. falciparum* sporozoites and crosslinked with dithio-bis-succinimidyl propionate (DSP). The crosslinked sporozoites were washed until no further label could be released, then incubated with DTT to reverse the covalent crosslinking. Radiolabelled proteins released by reduction were separated by SDS-PAGE and autoradiographed. Two bands of 55 kD and 20 kD proteins were detected. No such peptides were found in parallel experiments using rat hepatocytes (not invaded by *P. falciparum* sporozoites). Both electrophoretically purified 20 kD and 55 kD proteins inhibited the binding of radiolabelled 20 kD and 55 kD proteins to *P. falciparum* sporozoites and reduced the invasion of sporozoites in an in vitro assay. Invasion was also inhibited by plasma membrane proteins prepared from human liver but not from rat liver or kidney. We propose that these 55 kD and 20 kD proteins represent putative human hepatocyte receptors for *P. falciparum* sporozoite invasion.

SPECIFIC ANTIGENS OF *ONCHOCERCA VOLVULUS*

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Chronic infection with the filarial parasite *O. volvulus* cause dermatologic and ocular complications including blindness. The skin biopsy technique used to diagnose *O. volvulus* infection is unable to detect individuals with very low levels of infection, particularly in the prepatent stage of the disease. To be able to detect such infections is of utmost importance during epidemiological investigations and follow up studies in areas of intensive control and repopulation programmes. Over the years many immunodiagnostic procedures have been developed to replace the parasitological method of diagnosis, but the frequent presence of cross-reactivity with other filarial species render the assays unfit in areas where several human filariae are simultaneously present or where filariae are common parasites of animals with which man is in contact. To study if the development of specific immunological assays is possible in onchocerciasis the sera of 40 onchocerciasis patients, 34 endemic controls and 22 filariasis bancrofti patients were investigated with the Western Blot method.

The results suggested that highly specific immunological assays could be developed for the detection of onchocerciasis infections. Especially a 16 kD antigen is promising for further development. The sensitivity of the immunological assays is probably equal to the parasitological method of diagnosis (skin snip).

MODIFICATION OF *ONCHOCERCA VOLVULUS* MICROFILARIAL CUTICLE BY ENZYMATIC DIGESTION.

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Intact microfilariae of *Onchocerca volvulus* are thought to be non-immunogenic because of their inability to induce immune-reactions in or near their site of location (i.e. the eye and the skin). They show no staining in an indirect immunofluorescence test (IFAT) using pooled patient serum. Not until they degenerate do they provoke an immune-response, a situation in which antigen becomes exposed to the immune system.

Because of the observation that intra-uterine juvenile microfilarial stages do show staining in IFAT, we assumed that the microfilarial cuticle is in fact immunogenic and that this immunogenicity is diminished by intra-uterine masking of surface markers.

We obtained intra-uterine mature microfilariae and tried to unmask antigens using several different agents. We found that enzymatic treatment of the outer surface layer with pepsin results in positive staining in IFAT which means they become immunogenic.

The results suggest that intra-uterine masking of immunogenic cuticle components is an important step in the development of *O. volvulus* microfilariae.