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Geachte Professor de Geus,

Bijgaand treft U 4 abstracts aan die behoren bij voordrachten,
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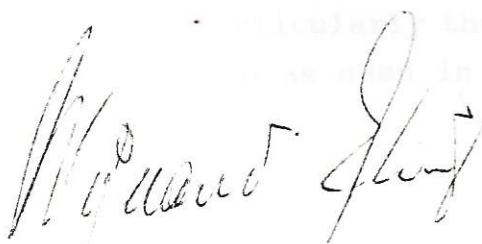
Ik wil U vragen of TGM deze abstracten zou willen publiceren
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U dankend voor de te nemen moeite, tekent.

Met vriendelijke groet,

Wijnand Eling, secretaris NVP



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PHOSPHORYLCHOLINE AND THE DETECTION OF TOXOPLASMA ANTIBODIES
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Diagnosis of an infection with Toxoplasma gondii largely depends on the detection of specific antibodies. Low antibody titers can be found in sera of individuals lacking other evidence of infection. These naturally occurring or induced antibodies to toxoplasma antigens are mostly IgM and are only occasionally found to be of the IgG class. We observed that preincubation of these sera with 1 mM of a quaternary ammonium compound such as phosphorylcholine chloride (PCC) caused the optical density readings (OD_{450}) of the indirect enzyme linked immunosorbent assay (ELISA) for IgM drop to background values. The values obtained with sera from established cases of acute toxoplasmosis were not or only slightly affected. The effect of preincubation with PCC was investigated with sera of specific patients, 263 non-selected sera obtained from women during pregnancy and 560 non-selected sera from healthy blood donors. The same sera preincubated with 1 mM myo-inositol did serve as controls. The drop in ELISA-IgM OD_{450} depended on the individual serum and the effect proved to be highly reproducible. The reductions found were from 0 till 80 percent. The results obtained for the groups of sera investigated did not differ. Evaluation of the antibody response in immunoblots for IgM revealed the disappearance or diminished staining of particularly the 20, 17 and 14 kD bands. The response to 6 kD antigen as seen in primary infections did not change.

OXIDATIVE KILLING IN TWO INCOMPATIBLE TREMATODE-SNAIL COMBINATIONS

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Molluscan haemocytes may exert cytotoxicity through generation of reactive oxygen intermediates (ROIs) in analogy to the mammalian respiratory burst. Generation of ROIs by haemocytes a) follows kinetics similar to those of mammalian leukocytes during respiratory burst and b) is sensitive to catechol-like phenols, specific blockers of the assembly of the NAD(P)H-oxidase responsible for oxidative activities in mammalian leukocytes [1,2]. The effect of the catechol-like phenol protocatechuic acid (20µg/ml) on the outcome of encounters between sporocysts of incompatible trematodes parasites and snail haemocytes was investigated to assess ROI-involvement in haemocyte-mediated killing.

Schistosoma mansoni and *Trichobilharzia ocellata* sporocysts were incubated *in vitro* with haemocytes of incompatible snail species (*Lymnaea stagnalis* and *Biomphalaria glabrata* respectively). *L. stagnalis* haemocytes were obtained from prodding-blood, blood was taken from *B. glabrata* by heart puncture, the haemolymph was diluted 3-fold in sterile snail saline.

Using an inverted microscope, sporocyst survival time was determined (criteria; motility and flame cell activity). Death of *S. mansoni* sporocysts encapsulated by *L. stagnalis* haemocytes occurred after ca. 16h, inclusion of protocatechuic acid resulted in 6-8h longer survival time of sporocysts. Similar effects of protocatechuic acid were observed in *T. ocellata*/*B. glabrata* interactions although less evident; sporocyst killing in control occurred only after ca. 48h and contaminating micro-organisms were present in the assay. Concentration of *B. glabrata* haemocytes in anti-clumping buffer to obtain a similar cell density as observed in *L. stagnalis*, yielded monocellular suspensions of haemocytes that did adhere to but could not kill *T. ocellata* sporocysts.

This suggests that ROIs do take part in killing of sporocysts but that they are not essential for elimination of this multicellular target. Haemocyte-mediated toxicity must therefore also comprise other effector mechanisms.

References

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HOW DIGENETIC TREMATODES INTERVENE IN THE NEUROENDOCRINE CONTROL OF REPRODUCTION OF THEIR INTERMEDIATE HOSTS, FRESHWATER SNAILS

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The combination of the avian schistosome *Trichobilharzia ocellata* and the freshwater snail *Lymnaea stagnalis* is used as a model system to study neuro-endocrine interactions between trematode parasites and their intermediate hosts.

Snails infected at a juvenile stage show a reduction or cessation of fecundity. The occurrence of schistosomin, a peptidergic agent in the haemolymph of infected snails, might explain the reduction of fecundity in trematode-infected snails. Schistosomin interferes with the action of the snail's female gonadotropic hormones upon their target organs, parts of the reproductive tract. This effect is exerted at the hormone-receptor level. Schistosomin appeared to be a neuropeptide derived from the CNS of the snail and it could be purified and partially characterized with biochemical techniques. The peptide has a pI > 6, a molecular weight of \pm 8.7 kD and consists of 79 amino-acids. Purified schistosomin inhibits the effects of different gonadotropic hormones upon their target organs at the level of the receptor complexes which indicates that only one type of schistosomin is involved. At the moment the structure of the molecule is under further investigation with biochemical and recombinant-DNA techniques.

The presence of schistosomin in haemolymph of infected *L. stagnalis* has appeared to be a general phenomenon in snail species infected with digenetic trematodes, species that are intermediate hosts for parasites causing schistosomiasis included. However, schistosomin is genus-specific: it only has effect when gonadotropic hormones and schistosomin are derived from species belonging to one and the same genus.

Schistosomin is not produced by *T. ocellata*. The parasite induces the release of schistosomin from the brain of its host. A special *in vitro* bioassay was developed to study which parasitic stage (or factor) induces the release of schistosomin from the CNS of the snails. In this bioassay 200 CNS of *L. stagnalis* are incubated in HEPES buffered saline with extracts of parasitic stages. After incubation, the incubation medium is HPLC analysed for the presence of schistosomin. It was demonstrated that synthesis and release of schistosomin is induced by a humoral factor, which is derived from developing cercariae. Studies are now in progress to establish the nature of this cercarial factor involved in the release of schistosomin.

SNAIL-SCHISTOSOME INTERACTION: AN ASSAY FOR HAEMOCYTE RESPONSES TO
LARVAL SURFACE DETERMINANTS

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Schistosome-snail immunological compatibility is, in part, determined by the molecular composition of the parasite's tegumental surface. Of the many determinants exposed at this surface each may play a different role in the interaction with haemocytes, the defence cells of the snail host. In order to elucidate which of the determinants find a receptor on the haemocyte and are bound to, which of these subsequently activate the haemocyte to cytotoxic activities and which ones do not, and which ones are not reacted to at all, an assay is needed that allows to study haemocyte responses to individual surface determinants. The development of this assay is the aim of the present study. The compatible combination of the avian schistosome Trichobilharzia ocellata and its snail host Lymnaea stagnalis was used as a model. Different methods were applied to collect determinants from cercariae and from in vitro cultured mother sporocysts: extraction with mild detergents, freezing-thawing, mechanical shearing. None of these yields a fraction of solely surface determinants, however. The parasitic (surface plus somatic) determinants were separated by electrophoresis. For a subsequent haemocyte binding assay with the separated determinants, electrophoresis must be performed in FMC ProSieve agarose: this matrix is neutral to haemocytes (these are neither repelled nor bound by it) and it is translucent, allowing microscopic observation. A suspension of haemocytes was layered over the agarose gel containing the separated determinants. Haemocytes do bind to some bands, not to others. Results are not consistent, because they are still too much influenced by experimental variables (especially the chemicals used to fix the determinants to the agarose gel). Once optimal conditions are found the assay will be used to investigate the nature of haemocyte-binding and non-binding determinants, and the state of activity of bound haemocytes.