46. Immunology-7 (Trypanosomosis/Leishmaniosis)

O-0515

USE OF TUBULIN FOR IMMUNIZATION AGAINST TRYPANOSOMIOSIS.

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The immunotherapeutic potential of tubulin against trypanosomiosis was investigated. A native tubulin enriched protein (NTP), was purified from *Trypanosoma brucei brucei* and used for immunizing mice or rabbits. Synthetic peptides (STP) based on the C-terminal of the ß-tubulin cDNA of *T.b. rhodesiense* were also used. The NTP induced protection in mice challenged with *T.b.brucei*. No protection was observed with the STP. The ability of the rabbit anti-NTP or anti-STP sera to inhibit proliferation of trypanosomes was investigated using *T.b.brucei* in culture. The anti-NTP strongly inhibited the proliferation of the trypanosomes. The anti-STP also inhibited proliferation but was much less potent than the anti-NTP. It could not be established why the STP could not confer some protection in mice. Nevertheless these data suggest that trypanosome tubulin may serve as a specific immunotherapeutic target against trypanosomiosis.

O-0516

NITRIC OXIDE PRODUCTION IN VERVET MONKEYS INFECTED WITH *TRYPANOSOMA RHODESIENSE*: A RETROSPECTIVE STUDY

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In murine trypanosomosis, increased nitric oxide (NO) production has been shown to play a significant role in immunosuppression and other pathological conditions such as anaemia. In this study, the vervet monkey (*Cercopithecus aethiops*), model of Rhodesiense sleeping sickness was used to study NO production. Serum and cerebrospinal fluid (CSF) samples were obtained from ten monkeys infected with *T. b.rhodesiense* KETRI 2537 and were assayed for nitrate. Before infection, no nitrate was detected in CSF but in serum a background concentration of approximately 62.4µM±1.84 was obtained. Following infection, the serum nitrate concentrations increased rapidly with a peak at day 28 (216µM±3.92), thereafter decreasing to pre-infection levels by day 42. In CSF, NO levels had a similiar trend although the values were lower. The NO peak corresponded to peak parasitemia, low packed cell volume (PCV) and high body temperature. This study showed that NO production is increased during trypanosomosis infections with a strong correlation with the clinical disease. Futher investigations are being carried out to generate information useful in designing appropriate treatment strategies in the management of Human African Trypanosomosis.

O-0517 THE IMMUNOPATHOGENESIS OF *LEISHMANIA DONOVANI* INFECTION IN *Nramp1* CONGENIC MICE

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The Natural resistance associated macrophage protein gene (*Nramp1, Ity/Lsh/Bcg*) regulates macrophage activation for antimicrobial activity. To investigate the mechanisms by which *Nramp1* influences susceptibility to intracellular infection, *Nramp1* B10.L-Lsh^r (N20) resistant and B10 susceptible congenic mice were infected with *Leishmania donovani* amastigotes in duplicate experiments.

Fifteen days post infection the hepatic parasite count was more than one log unit greater in susceptible than resistant mice. Light microscopy revealed morphological changes in the Kupffer cell population within 24 hours of infection in resistant but not susceptible mice. Fifteen and 30 days post-infection, hepatic granulomas were significantly more numerous in susceptible animals. RNA was extracting from livers harvested during early infection and semi-quantitative RT-PCR was used to study changes in mRNA expression of murine inducible nitric oxide synthase (iNOS), interleukin-12 p40 subunit (IL-12), the neutrophil attractant chemokine KC, *Nramp1* and the housekeeping gene GAPDH. This revealed a biphasic up-regulation of iNOS, IL-12, KC and *Nramp1* mRNA expression relative to GAPDH in resistant and susceptible animals following infection. Early iNOS and KC expression were significantly greater in resistant than susceptible mice, consistent with previous *in vitro* studies of the innate immune response in transfected cell lines. By day 15, the adaptive immune response was associated with significant induction of iNOS and KC mRNA levels in both resistant and susceptible mice.

These results suggest that nitric oxide mediated parasite killing contributes to the innate immune response in *Nramp1* resistant animals but is deficient in *Nramp1* susceptible mice.

O-0518 ATNI-LEISHMANIAL ACTIVITY OF MURINE MACROPHAGES STIMULATED WITH NERVE GROWTH FACTOR

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Although nerve growth factor (NGF) is a well known neurotrophic polypeptide necessary for the normal development and function of sympathetic and sensory cells, recent findings have shown that NGF regulates immune and inflammatory responses through direct effect on immunocompetent cells including macrophages. Therefore, we investigated the possible effect of NGF on anti-leishmanial activity of murine peritoneal macrophages. NGF enhanced killing of *Leishmania donovani* promastigotes by macrophages. In the presence of various doses of NGF, macrophages showed the increased production of H_2O_2 in a dose dependent manner, but not NO_2. The anti-leishmanial activity and H_2O_2 production induced by NGF were inhibited by the addition of glutathione peroxidase, a H_2O_2 inhibitor, but not L-N^G-monomethylarginine, a NO inhibitor. Thus, these results suggest that NGF may act as a bioactive cytokine to promote anti-leishmanial activity of macrophages through the killing process dependent on H_2O_2.