

CHAPTER VI

DISCUSSION

In vitro maintenance of antigen preparation

Studies on the immunization of laboratory animals against *Ascaris* infections have been done by many investigators (see art I). However the results of these studies never led to an accepted opinion that particular antigens somatic or excretory/secretory in nature were functional antigens. Most promising results were obtained with antigens derived from larval stages *Ascaris suum* where particularly molting fluid released between the L3 and L4 stage induced protective immunity. On the other hand ES antigens from the L2 stage also could produce protective immunity provided that the culture was maintained longer than two weeks. Particularly the maintenance of L2 larvae in culture medium without serum caused problems with regard to the survival of the larvae. The introduction of glycyl-L-histidyl-L-lysine acetate tetrahydrate (Stromberg et al., 1977) in the culture medium as a substitute for serum made longer in vitro cultures possible. In this study both ES antigens from L2 and a mixture of L3 and L4 larvae were prepared. For comparison somatic antigen was also prepared from the same larvae after the in vitro maintenance of several weeks, or separately from directly derived larvae.

The rise of inducing allergenic reactions due to the application of unpurified *Ascaris* antigens is a well-known phenomenon. In one of the experiments, therefore, only ES antigens were tested separately and in combinations. The latter was done to study the theoretical possibility of a

synergistic effect. Further more, also extra control animals were included to study the possible effect of Freund's adjuvant on the migratory behavior of the larvae. In one experiment (IV) a successful study was undertaken to exchange *A.suum* for *Ascaris lumbricoides*. A good cross protection was observed when *A.suum* antigens were used for immunization. This makes future experiments easier to plan when a vaccine for human purpose is envisaged.

Serology


With regard to the serological results the most surprising result is the obvious stage specificity of the antibodies induced with ES antigens of L2 and L3/4 respectively. In Experiment II this was less pronounced but in the later experiments it was clearly observed. Probably the quality of the hatches of ES antigens used in the consecutive experiments had improved, i.e. less somatic antigen in the culture medium due to dead deteriorating larva. Cross reaction between stages with somatic antigens were observed whenever they were used. Interesting was also the specificity of sera antibodies induced by ES/L2 *A.suum* antigen which is shown in Table 10. Virtually, no cross reaction occurred with antigen of *A.lumbricoides*. The somatic antigen of L2 larvae used in experiment II for immunization apparently was of bad quality since it provoked a weak antibody response only after three injections. Nevertheless significant protection was observed in this group indicating that antibodies alone are not sufficient to inhibit migration of larvae.

Protection

In order to be able to study the protective properties of these antigens, first an animal (mouse) model was worked out. The migratory behavior of larvae in experimentally infected mice (2000 embryonated eggs, orally) showed

two distinct peaks for recovery of larvae exclusively from liver and lungs at days 3 and 7 after infection respectively. This is in accordance with results obtained by most investigations in literature even when other animals were used like guinea pigs or rabbits (OA. Bindseil, 1969). It was remarkable that in the non-protected animals the recovery of migrating Larvae is so limited (Table 2,3). In the Experiments II, III and IV, very reproducible results were observed when mice were immunized with ES antigens of L2 *A.suum* larvae. An average of 90 % reduction of migrating larvae even in the liver suggests that inhibition or trapping occurs at a very early stage of infection. In Experiment II it low infection rate in the control group, which was killed at day 49 of the immunization, was very likely due to unwanted failure with the infection dosage. In experiment III relatively many animals were totally protected (no larval counts) which inevitably influenced the average numbers. Few animals did show more migrating larvae than was expected (Table 5) and were at least less protected than the average percentage of reduced number of migrating larvae suggests. Due to the presence of one obviously non-infected mouse in both control groups the statistical analysis (Wilcoxon's test) gave poor results in Tables 5 and 9. The relative lower counts for migrating larvae in immunized and control groups when challenge infections were given with *A. lumbricoides* eggs is an indication that the infectivity of the eggs, obtained from hospital may have been reduced because of the effects of drug treatments. However, the ES antigens derived from L2 larvae from these eggs did produce sufficient immunity. Although somatic larval antigens significantly reduced the number of migrating larvae too, experiments II and IV were only carried out with ES antigens. The goal of finding functional protective antigens is shared with the wish to find a minimum number of purified antigens. In the immunoblotting results it is shown that the antibodies in the sera of the mice protected with ES/L2 *A.lumbricoides* antigens reacted with the ES *A.suum* antigens from both L2 and L3/4 in the homologous system they recognize a single antigen of

molecular weight 46 KD, which on the contrary was not recognized by sera from mice protected with ES/L2 *A.suum* antigen. It is not clear why comparable combinations in figures 5 and 6 do not result in similar reactions. This may be due to quality of the sera used in the various experiments. Therefore, no definite conclusions can be drawn from these figures. The ES antigens recognized however are very close in molecular weights but certainly different as simultaneous blotting has shown and are likely the main antigens which induce the protection observed when a challenge infection was given with both *A.suum* and *A.lumbricoides*.



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