Discussion

S. stercoralis is endemic in tropical and subtropical zones. Cases are usually asymptomatic, but the progression of such asymptomatic or mildly symptomatic infections to fatally hyperinfected stage is a well-documented complication of immunosuppression. There can be no doubt that the parasitological confirmation of light to moderate infection with the parasite is frequently difficult (Sato *et al.*, 1985). L1 are the stage that is frequently found in the feces but the eggs, adult, and L3 are rarely found in it. The 41-kDa protein is important for study in *S. stercoralis* larvae because it is one of the most prominent bands detected by human sera with strongyloidiasis. It has been reported that the 41-kDa protein is recognized by IgG in 91% of sera from patients with confirmed strongyloidiasis and 100% of sera from patients with suspected strongyloidiasis by Western blotting (Conway *et al.*, 1993b). However, because it is difficult to culture L1 in an amount adequate for antigen detection, the antigen is usually obtained from L3 that are easier to culture (Neva *et al., 1981*; Gam *et al.*, 1987; Sato *et al*, 1990; Siddiqui *et al.*, 1997). Although this study has the problem of culturing L1 in large numbers, our study showed that the 41-kDa protein was found in L1, L3 and adult worms. The protein at the molecular weight of 41-kDa purified from L3 was chosen to use as immunogen for the production of mAbs.

Coprologic methods, such as direct smear and formalin-ether concentration methods, have low sensitivity due to the low larval density in feces. The agar plate culture seems to solve this problem, and although it is highly specific and sensitive for

confirmation, it is a time consuming to be suitable in routine laboratories (Koga *et al.*, 1990; de Kaminsky, 1993; Sato *et al.*, 1995). Immunodiagnostic methods are reportedly more sensitive than coprologic methods but they cannot differentiate between an ongoing and a past infection. For the past several years, there have been several reports on the use of sandwich ELISA to successfully detect coproantigen such as in rats experimentally infected with *S. ratti* (Nageswarran *et al.*, 1994); in dogs experimentally and naturally infected with *Echinoccocus granulosus* (Malgor *et al.*, 1997), and in humans with *Taenia*, *Fasciola hepatica, O. viverrini, Echinococcus* or *E. histolytica* infections (Allan *et al.*, 1992; 1993; Espino and Finlay, 1994; Sirisinha *et al.*, 1995; Abdel-Rahman *et al.*, 1998; Espino *et al.*, 1998; Singh *et al.*, 1999). In this study intended to evaluate the coproantigen detection of *S. stercoralis* antigens in feces using the capture ELISA method.

The first attempt in this study was to produce mAbs against the 41-kDa protein. The mAbs would be used in the sandwich ELISA. The production of mAbs against the 41-kDa protein seems logical since the protein appears to be present in L1 and adult stages, which are normally found in the intestine of infected individuals. However, this study failed to identify hybridoma clones that secreted antibodies specific to the 41-kDa protein in three fusion experiments. The reason why mAbs to 41-kDa protein could not be obtained is not clear. Finally, this study was able to produce four mAbs derived from spleen cells of a mouse immunized with crude antigens of L3. They consistently reacted with L3 antigen by ELISA and were later used for coproantigen detection.

About the coproantigen detection, it is necessary that the test be sensitive and specific enough to detect antigens in the fecal samples that contain lots of other

components. The test should not depend on the physical integrity of the organisms and should allow the rapid processing of large numbers of samples during epidemiological surveys. It is very difficult to produce a mAb that is highly specific and does not cross-react with the multitude of fecal components. However, this study did not succeed in developing a sandwich ELISA that could detect *S. stercoralis* antigens in the feces of strongyloidiasis cases. Our tests could not distinguish the fecal samples of infected individuals from those of uninfected individuals. There are a few explanations for this. First of all, the amount of antigens in infected feces may be so low that the signal generated by antibody recognition was not above the background level. Chaicumpa *et al.* (1992) and Sirisinha *et al.* (1995) experienced the same problem of coproantigen detection in patients infected with *O. viverrini.* They explained that the mAb used in the system may be detecting antigens in the feces that were not sufficient in quantities. An important point is that this study is trying to detect antigens that are shed or secreted into the feces. These shed or secreted antigens may not be equivalent to those in crude antigens of L1 and adult prepared in laboratory. It is therefore possible that the mAbs do not react with antigens present in infected feces. Unfortunately, there is still no information regarding the kinds of *S. stercoralis* antigens found in the feces of infected individuals. An alternative explanation for the inability of the sandwich ELISA to distinguish between infected and uninfected feces is that the antibodies may be reacting nonspecifically with the fecal components leaving very little antibodies to react with the antigens. Another consideration for the problem that this study could not detect antigens of *S. stercoralis* in the feces is that there might be interfering materials in the fecal samples (Hanvanich *et al.*, 1985). These materials may cause denaturation, proteolysis or

desorption of reactants on the solid phase surfaces leading to decreased reactivity in the ELISA. In the study of Viscidi *et al.* (1984), they explained that the feces contained an activity of proteolytic enzyme which desorbed the immunoreactant from solid phase surfaces and reduced the sensitivity of ELISA system. This desorption could be solved by using 50% FCS, acid buffer (0.1 M citrate buffer, pH 5.0) containing 5% BSA or adding protease inhibitors into the feces. The acid buffer was intended to decrease the activity of pH-specific intestinal proteases and BSA was added to provide an excess of substrate (Chaicumpa *et al.*, 1992). However, it showed low sensitivity as compared to using 50% FCS. The active factor in FCS preventing desorption is unknown, however it contains several types of protease inhibitors. In addition, its high protein content might provide a competing source for the substrate. The protease inhibitors such as PMSF and soybean trypsin inhibitor were much less effective than 50% FCS and acid buffer (Viscidi *et al.*, 1984). However, when the cocktail of protease inhibitors (PMSF and EDTA) were used, it showed high sensitivity and specificity (89% and 100%, respectively) when tested for coproantigen detection in amebiasis patients.

One would think that using two or more specific mAbs should increase the signal and hence improve the sensitivity of the sandwich ELISA for coproantigen detection. In the present study, this study combined two mAbs (clones 20 and 65), either as the capture or detecting antibodies, but this combination did not seem to help improve the signal. In the study of *O. viverrini* infection, the use of a cocktail of three monoclonal antibodies for coproantigen ELISA gave lower sensitivity than coprologic method (Sirisinha *et al.*, 1991). It would have been helpful also if this study had produced mAbs against the 41-kDa protein. This protein has been shown to be quite

immunogenic in human strongyloidiasis (Conway *et al.*, 1994). A mAb or a panel of mAbs to the 41-kDa protein would be interesting to test whether they could be useful for coproantigen detection by the sandwich ELISA.

Additional work that could be done in the future is to test these mAbs for coproantigen detection by competitive ELISA. The competitive ELISA is very specific, although it loses some of the sensitivity typically associated with ELISA. It may be useful when the sample is very dirty or when there is a high degree of cross reaction in the samples.

In conclusion, this study was successful in producing four mAbs against crude L3 antigens of *S. stercoralis*. The mAbs reacted strongly with L3 antigens by ELISA but they did not show specific banding patterns with L3 antigens by Western blot analysis. However, in this study failed to obtain mAbs to the 41-kDa protein of *S. stercoralis*. The use of mAbs as the capture antibodies and rIgGαL3 antigens as the detecting antibodies, or vice versa, in the sandwich ELISA could at least detect crude antigens of L3 and L1. The application of the system to detect *S. stercoralis* antigens in infected feces, however, did not give the signal above that given by uninfected feces. It was hypothesized that the amount of soluble antigens in the feces may be too low in quantities to be detected by the test and/or the antibodies used in the system may be reacting with the fecal components giving the nonspecific signal that overshadowed the specific reactivities.