Trichinella spiralis: the infectivity of synchronous newborn larvae of different ages inoculated intraocularly

Abstract Trichinella spiralis infection results in the transformation of muscle cells into a new, non-muscular cell called the nurse cell, and the nurse cell-muscle larva complex is finally created. To investigate whether T. spiralis infectivity is NBL age-dependent, five groups of synchronous newborn larvae (sNBL) were obtained at 1, 9, 24, 48, and 72 h of age and were inoculated into mice by intravenous injection into the retro-orbital venous plexus. When both “young” groups of sNBL (1 and 9 h old) were injected, the highest number of larvae were capable of infecting the muscle cells. The highest infectivity of 80.0% was observed for 9-h-old sNBL. In older sNBL the infectivity gradually decreased; thus, for 72-h-old sNBL the lowest level – 0.1% – was detected. Therefore, an “age limit” for NBL infectivity in the present study was precisely determined.

Introduction

Trichinella spiralis is a unique nematode, e.g., as it spends its larval and adult life in the same host and both the muscle larva and the adult parasite occur as intracellular stages. The penetration of NBL into the muscle cell causes significant structural and biochemical changes within the place of deposition. It has been demonstrated that NBL are capable of transforming the muscle cell into a new, functional cell possessing a nonmuscular nature, which has been called the “nurse cell” Purkerson and Despomier 1974, and this process is referred as basophilic transformation (Gabryel and Gustowska 1967, 1970).

Infection with T. spiralis stimulates a complex, stage-specific immune response in mammalian hosts (Bell et al. 1979; Ahmad et al. 1991). Host immunity to NBL has been demonstrated in both in vitro and in vivo experiments. The in vivo studies have been based on immunization with NBL, or their antigenic products, followed by an intravenous challenge with NBL (Wang and Bell 1988a). The in vitro analyses have shown that NBL of different ages display distinct cuticular antigens, differ in their infectivity to the host, vary in their resistance to cytotoxicity (Binaghi et al. 1984), and attract different populations of host leukocytes to the larval cuticle after in vitro incubation (Wakelin and Donachie 1983; Zhu and Bell 1990).

The infectivity of NBL of different ages given intravenously has been studied in various mouse and rat strains. The results demonstrated that NBL possessed similar degrees of infectivity for up to 24 h of age (Wang and Bell 1988b); however, another study showed the low infectivity of 2-h-old NBL and the high infectivity of 20-h-old NBL in outbred Swiss mice and the adherence of eosinophils mainly to 2-h-old NBL, whereas macrophages adhered to 20-h-old NBL only (Binaghi et al. 1984; Gansmüller et al. 1987). Our earlier experimental data demonstrated an “NBL age limit” for NBL infectivity; however, until now this has not been precisely determined (Wranicz et al. 1988).

The aim of the present study was to examine (1) the infectivity of sNBL of different ages and (2) the new route of intravenous injection, i.e., the retro-orbital venous plexus.

Materials and methods

Collection of newborn larvae

Adult worms were recovered from the small intestine of Swiss mice infected per os 7 days previously with 700 muscle larvae each. The
sNBL were collected using a method described elsewhere (Wranicz et al. 1998). After 2 h of incubation, NBL were divided into five groups of sNBL. The sNBL of group I, 1-h-old sNBL (age \(1 \pm 1\) h), were used immediately. All other groups were maintained for longer periods and were marked as 9-h-old sNBL (age \(9 \pm 1\) h; group II), 24-h-old sNBL (age \(24 \pm 1\) h; group III), 48-h-old sNBL (age \(48 \pm 1\) h; group IV), and 72-h-old sNBL (age \(72 \pm 1\) h; group V).

Experimental animals

A total of 20 male Swiss mice aged 3 months were used in the experiment. Mice were divided into five groups, and all were given sNBL by injection into the retro-orbital venous plexus. The number of sNBL inoculated per mouse was as follows: group I, 8,187–13,167; group II, 3,000–17,470; group III, 2,650–10,500; group IV, 3,800–12,960; and group V, 5,126–5,700. All mice were necropsied at 8 weeks after infection. The carcasses were minced and incubated individually in standard pepsin-HCl digestion fluid, and the total numbers of muscle larvae per individual were calculated. The infectivity was calculated as the ratio of the infective dose of sNBL to the total number of ML recovered per individual. Differences in the infectivity of sNBL among groups were determined by the non-parametric Mann-Whitney-Wilcoxon test. Correlations between the infective doses of sNBL and the intensity of infection in all groups were evaluated by Pearson’s correlation coefficient.

Results

The results of the experiment show that sNBL aged up to 72 h have different degrees of infectivity. The percentages of inoculated sNBL recovered as ML are reported in Table 1 and Fig. 1. The highest numbers of ML were recovered from mice inoculated with 1- and 9-h-old sNBL (infectivity 62.3% and 80.0%, respectively). For 24-and 48-h-old sNBL a decrease in infectivity was observed and the percentage of recovered ML was much lower. For 72-h-old sNBL a trace level of infectivity amounting to 0.1% was documented. Differences in numbers of recovered ML among all experimental groups were significant. Statistical analysis showed nonsignificant correlations between the infective doses of sNBL and the intensity of infection in all groups.

Discussion

During the intestinal phase of Trichinella spiralis infection the adult worms shed NBL, which penetrate the intestinal mucosa and pass to striated muscles after circulating mainly through the bloodstream (Wang and Bell 1986).

It has been well documented that only NBL are capable of infecting and basophically transforming the muscle cell (Gabryel et al. 1985). A number of studies have reported that the infectivity of T. spiralis NBL is limited. After intravenous injection of NBL of any age up to 24 h, similar numbers of ML were recovered (Wang and Bell 1988b). It is difficult to compare these results with ours because the NBL used in the above-cited report were not synchronous. Another study has shown differences in the percentage of ML recovered after intravenous injection of 2- and 20-h-old NBL (infectivity into the tail vein 10% and 50%, respectively; Binaghi et al. 1984).

The present study reveals that the infectivity of NBL inoculated into mice by intravenous injection into the retro-orbital venous plexus is NBL age-dependent, reaching a maximum in 0- to 10-h-old NBL and ranging from 62.3% to 80.0%. Moreover, the present results did not confirm the low infectivity of 2-h-old NBL (10%) reported by Binaghi et al. (1984). We suppose that the high degree of infectivity observed in our study, which was more than 6-fold higher, could be the result of different routes of inoculation as well. Our earlier experiments have demonstrated a high transformation potential for "young" sNBL. Furthermore, the transformation potential seems to be age-dependent. When 1- and 9-h-old sNBL were injected intramuscularly, basophilic transformation of muscle cells was observed, whereas after the inoculation of 6-day-old sNBL, none was found (Wranicz et al. 1998).

<table>
<thead>
<tr>
<th>Group</th>
<th>Infectivitya</th>
<th>Infective doseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>62.3*</td>
<td>9,847.0 ± 2875.2</td>
</tr>
<tr>
<td>Group II</td>
<td>80.0*</td>
<td>10,556.7 ± 7,256.4</td>
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<tr>
<td>Group III</td>
<td>43.5*</td>
<td>7,517.5 ± 3,474.1</td>
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<tr>
<td>Group IV</td>
<td>20.9*</td>
<td>8,420.0 ± 4,580.5</td>
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<tr>
<td>Group V</td>
<td>0.09*</td>
<td>5,317.3 ± 280.1</td>
</tr>
</tbody>
</table>

* \(P < 0.05\)

In% Data represent mean values ± SD