Membrane fluidity on the surface of *Hymenolepis diminuta* (Cestoda): immunological implications

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SUMMARY

Regional differences of *Hymenolepis diminuta* to immune lysis have been investigated by monitoring surface membrane fluidity utilizing fluorescence recovery after photobleaching (FRAP). Although the surface membrane of the newly excysted stage was completely immobile the molecular fluidity of the strobila membrane of 4-day-old parasites was greater than that associated with the scolex/neck region. Significant differences ($P > 0{\cdot}001$) occurred in the mobility of the strobila membrane of 4-day-old *H. diminuta* from 100-worm infections compared with 7- and 21-day-old parasites and 4-day-old individuals from 10-worm infections. Exposure to 50% normal rat serum and 1 mg/ml rat C reactive protein decreased or eliminated membrane fluidity. The significance of membrane mobility is discussed with reference to resistance to complement-mediated lysis and destrobilation.

Key words: *Hymenolepis diminuta*, membrane fluidity, destrobilation, complement, C reactive protein.

INTRODUCTION

Destrobilated worms have been the focus of much attention in studies on the rejection of *Hymenolepis diminuta* from both the rat, its natural host, and the mouse (Hopkins, 1980; Hindsbo, Andreassen & Ruitenberg, 1982). Destrobilation, thought to be a consequence of the immune response, increases in rats with high infection intensities (Hesselberg & Andreassen, 1975) and in secondary infections (Hesselberg & Andreassen, 1975; Andreassen & Hopkins, 1980). Destrobilation also occurs in nude rats, albeit more slowly, suggesting that T-cells are not essential for this process (Andreassen, 1981). It is still unclear, however, whether all worms destrobilate prior to expulsion or whether this is a potential escape mechanism, since 'destrobes' have the ability to undergo repair and regrow in immunosuppressed or naïve hosts (Hopkins & Stallard, 1976; Andreassen & Hoole, 1989).

In vitro studies have implicated complement in the killing of newly excysted worms and destrobilation of 4-day and older *H. diminuta* (Bögh, Christensen & Andreassen, 1986; Christensen, Bögh & Andreassen, 1986). Newly excysted and 2-day-old worms were lysed completely in 50% normal rat serum, whereas in 4 to 10-day-old worms, damage was always initiated some distance from the scolex and on some occasions the scolex was completely resistant to damage, suggesting survival of these worms as destrobes. The posterior tip of 7-day or older worms was also shown to resist complement damage and this correlated with the presence of expelled pieces of undamaged strobila in the faeces (Hindsbo *et al.* 1982). Resistance to complement-mediated lysis by other hymenolepid species differs from that observed in *H. diminuta* with newly excysted *H. nana* and *H. citelli* being lysed in sera from several mammals, whereas *H. microstoma* is also lysed in the sera of several mammals but not from its hosts, the mouse, rat and golden hamster (Bögh *et al.* 1986). The role of complement in mediating worm damage *in vivo* is not known. However, studies by Befus (1977) demonstrated the presence of C3 on the strobila of *H. diminuta* from both mice and rats. Schmidt & Ruppel (1988), however, concluded that complement-mediated lysis does not play a significant role during the rejection of *H. diminuta* from mice, since worms incubated in mouse sera were refractory to lysis despite the massive deposition of C3 on their surface.

Parce, Henry & McConnell (1978) and Taylor (1983) suggested that for complement-mediated lysis to occur the lesion-forming membrane attack complex must be integrated into the target membrane which thus requires a rapid lateral diffusion function in its lipid component. Studies on the lateral

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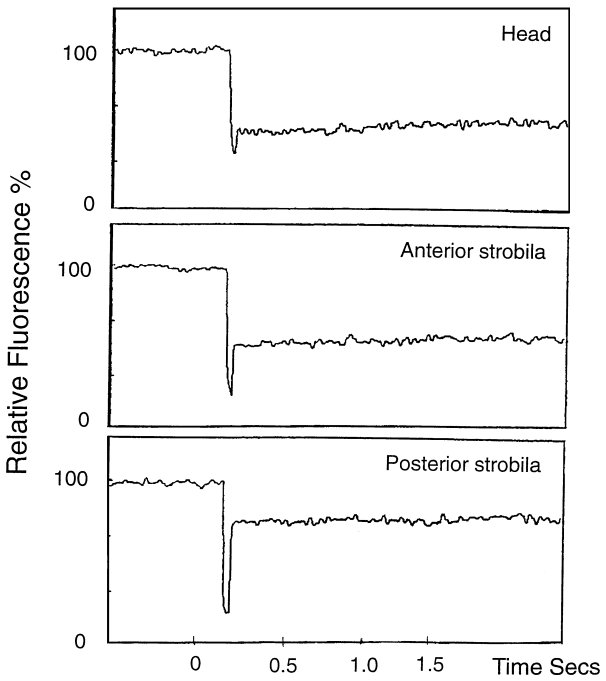

Fig. 1. Examples of the 'fast component' from FRAP curves of 4-day-old *Hymenolepis diminuta* showing increasing percentage recovery along the length of the worm.

diffusion of molecules in the surface membrane of *Schistosoma mansoni* have suggested that immobility of lipid molecules in the outer membrane of adult schistosomes may play a role in resistance to immune attack (Foley *et al*. 1986; Kusel & Gordon, 1989). In this study, species and regional differences in susceptibility of hymenolepids to immune lysis has been investigated using fluorescence recovery after photobleaching (FRAP).

MATERIALS AND METHODS

Infection and recovery of worms

H. diminuta were maintained in *Tenebrio molitor* beetles and male Wistar rats (bred at Keele University) as described by Taylor & Hoole (1997). Those used for experimentation were either obtained from beetles or from rats fed 10 or 100 cysticercoids via gavage.

Fluorescent labelling and recovery

Newly excysted, 4-, 7- and 21-day-old worms were incubated at room temperature (*ca*. 20 °C) for 2 min in 5-*N*-(octadecanoyl)-aminofluorescein (C_{18}FI) at a concentration of 10 μg/ml in M199 culture medium (Gibco) containing 5% heat-inactivated foetal calf serum (hiFCS). After rinsing in culture medium lacking hiFCS worms were transferred to carbamylcholine chloride (Sigma) in M199 (10 μg/ml) and mounted in cavity wells of silica gel or in a small plastic Petri dish containing culture medium. In addition, some 4-day-old parasites obtained from rats given 100 cysticercoids were incubated *in vitro* at 37 °C for 3 h in M199 containing either 50% normal rat serum, NRS (Christensen *et al*. 1986) or 1 mg/ml rat C reactive protein (CRP) isolated from serum as described by Taylor & Hoole (1997). Such treated worms were labelled as previously described. FRAP analysis was performed as described by Foley *et al*. (1986) with a 2 μm attenuated laser beam spot size and 100 msec bleach time. The percentage recovery and the lateral diffusion coefficient (D_L, rate of recovery) were determined and analysed using independent Student's *t*-tests.

RESULTS

Labelling of H. diminuta *with* C_{18}*Fl was extremely rapid and occurred within the 2 min incubation period*

Although the mean percentage recoveries of FRAP measurements taken on *H. diminuta* from 100-worm infections showed a differential membrane fluidity between the scolex/neck region and strobila, measurements on individual worms were extremely variable with regional differences in membrane fluidity occurring between the 2 regions and mobility or immobility of membranes occurring over the entire length of the worm. A further feature of some recovery curves of 4-day-old *H. diminuta* from 100-worm infections was the occurrence of a 'fast component' followed by a curve representative of an otherwise immobile membrane. The size of the fast component also varied along the length of the worm in its percentage recovery (Fig. 1). Cooling the worms to 4 °C decreased their recovery although it returned to previous levels as the temperature increased.

Membranes of newly excysted *H. diminuta* were shown to be completely immobile over their entire surface (Table 1), as were *H. microstoma* and *H. nana* (data not shown). Regional differences occurred between the scolex/neck region and the strobila of 4-, 7- and 21-day-old *H. diminuta* recovered from 100-cysticercoid infections. However, only in the 4-day individuals was this difference significant within the worm ($P > 0{\cdot}001$) and between the strobila of the 4-day-old parasites and 7- and 21-day-old worms ($P > 0{\cdot}001$). In contrast, in identically aged parasites from 10-worm infections there was no significant difference between the membrane fluidity of the scolex/neck and the strobila regions. However, there was a significant difference in the response of the strobila of 4-day-old parasites from 10- and 100-worm infections ($P > 0{\cdot}001$).

Incubation of 4-day-old *H. diminuta* from 100-worm infections in CRP for 3 h completely immobilized the surface membrane of the parasite with

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Table 1. Percentage recovery and lateral diffusion coefficient of the surface membrane of *Hymenolepis diminuta* at different ages, infection intensities and exposed to normal rat serum (NRS) and C reactive protein (CRP)

		Worm age post-excystment and number of cysticercoids administered						
			4 day					
Region of parasite	(%) Recovery and lateral diffusion coefficient (D_L)	Newly excysted: untreated	10 cysticercoids	100 cysticercoids			7 day: 100 cysticercoids, untreated	21 day: 100 cysticercoids untreated
			Untreated	Untreated	+50% NRS	+1 mg/ml CRP		
Scolex/neck	% Recovery (±S.E.)	0 (0)	22·5 (±3·1)	19·4 (±3·6)	30·3 (±4·1)	0 (0)	34·6 (±11·3)	47·3 (±3·1)
	$D_L \times 10^{-10}$ cm^2 (±S.E.)	0 (0)	596·5 (±188·7)	545·0 (±148·5)	30·7 (±9·1)	0 (0)	249·0 (±90·1)	356·7 (±106·0)
Strobila	% Recovery (±S.E.)	0 (0)	27·6 (±3·1)	52·7 (±4·74)	47·0 (±4·1)	0 (0)	89·9 (±1·3)	69·5 (±1·5)
	$D_L \times 10^{-10}$ cm^2 (±S.E.)	0 (0)	491·8 (±223·4)	1790·0 (±184·8)	35·2 (±7·2)	0 (0)	366·4 (±65·3)	542·0 (±152·2)
Vesicles	% Recovery (±S.E.)	—	—	—	18·4 (±3·4)	—	—	—
	$D_L \times 10^{-10}$ cm^2 (±S.E.)	—	—	—	6·1 (±1·5)	—	—	—
n =		10	10	40	10	10	15	10

percentage and rate of recovery being totally reduced (Table 1). Exposure of worms to 50% normal rat serum for 3 h, however, induced slow recovery in the membrane of the scolex/neck ($P > 0{\cdot}05$) and strobila ($P > 0{\cdot}001$) when compared with the corresponding region of untreated controls. In addition, surface vesicles formed by the action of the NRS had a significant reduction in the percentage and rate of recovery when compared to the scolex/neck region ($P > 0{\cdot}05$) and strobila ($P > 0{\cdot}05$).

DISCUSSION

The scolex and strobila of *H. diminuta* have previously been shown to differ in several respects, including resistance to complement-mediated damage (Christensen *et al*. 1986), complement-mediated adherence of leucocytes (Andreassen, Hoole & Befus, 1990), lectin-binding affinities (Schmidt, 1988), expression of gene products (Siddiqui, Karcz & Podesta, 1987) and the distribution of phosphorylcholine (Taylor & Hoole, 1997). Results presented here have also shown differences in lipid mobility between these two regions in 4-day-old *H. diminuta* from 100-worm infections where the mean percentage recovery in the scolex region showed that approximately 80% of the molecules were immobile, whereas in the strobila almost 50% of the molecules were free to move within the plane of the membrane and the recovery rate was approximately 3·5 times greater. Preferential labelling of the strobila of 4-day-old *H. diminuta* with merocyanin 540, a fluorescent compound that binds to lipids in a crystalline phase, substantiates these observed differences in lipid mobility between the 2 regions. In addition, C_{18}Fl labelling and FRAP analysis of 2 other hymenolepids, *H. microstoma* and *H. nana*, suggest that this regional difference may be a characteristic of *H. diminuta* (unpublished observations).

The assembly of the membrane attack complex of complement is thought to require a mobile lipid layer for insertion into the membrane to take place (Parce *et al*. 1978; Taylor, 1983). Foley *et al*. (1986) suggested that areas of restricted lateral diffusion in *S. mansoni* may be less susceptible to immune damage. The present study on 4-day-old *H. diminuta* from 100-worm infections would support this hypothesis, since the scolex/neck region has been shown to be resistant to complement-mediated damage (Bögh *et al*. 1986). Furthermore, the posterior proglottid, which is often resistant to complement damage, occasionally showed lipid immobility similar to that in the scolex. Expression of glycoconjugates on the surface of hymenolepid spp. has also been suggested to correlate with resistance to immunological attack. Schmidt (1988) revealed differences in the surface carbohydrates between the scolex and strobila of *H. diminuta*, with lectins specific for *N*-acetyl-glucosamine and galactose binding preferentially to the former region, whereas concanavalin A binding was restricted to the strobila. The fact that newly excysted parasites which are lysed completely in rat serum have complete membrane immobility and that *H. nana*, which has similar lipid mobility to the head of *H. diminuta* (unpublished observations), is lysed in rat serum suggests that susceptibility to complement-mediated damage may not simply be a consequence of restricted membrane fluidity and that other intrinsic and extrinsic factors may be involved.

Although mean values suggest that differences in lipid mobility do exist between the scolex/neck and strobila of *H. diminuta*, variability in lipid mobility exists within the population, which correlates with observed differences in susceptibility to complement-mediated damage *in vitro*; for example, only a proportion of 7-day-old worms survived as destrobilated worms (Christensen *et al*. 1986) and Bennet, Behm & Bryant (1990) noted differences in the metabolic properties between individuals. A further variable feature on several worms was the occurrence of a 'fast component' which, because it was not reduced during cooling, is probably a feature of the membrane rather than an experimental artefact. This phenomenon may represent a rapid lateral diffusion of a proportion of labelled lipids within an area of otherwise immobile membrane.

The increase in lipid mobility with age may be related to ontogenetic changes in membrane structure and/or function. Several studies have noted that the change in membrane permeability to carbohydrates and amino acids occurs during ontogeny of adult *H. diminuta* (see Arme (1988) for review). These, together with changes in other membrane functions, may clearly influence membrane fluidity or *vice versa*. The difference in membrane fluidity between the scolex/neck region and strobila of 4-day-old parasites noted in 100-worm infections was not observed in 10-worm infections. These differences may, therefore, be due to the parasite encountering hostile immunological attack or different nutritional interactions. In support of the former suggestion, worms incubated in complement showed a much slower rate of recovery than that of normal parasites, with the smallest values observed on damaged vesicles. Furthermore, membranes of worms incubated in CRP became completely immobile.

Whether or not complement damage is responsible for worm expulsion *in vivo* remains to be determined. Befus (1977) demonstrated the presence of C3 on the strobila of *H. diminuta* from both mice and rats, although Schmidt & Ruppel (1988) concluded that complement-mediated lysis was not a requirement for rejection of *H. diminuta* from mice as the parasite was resistant to mouse complement lysis, despite deposition of C3 on the worm's surface. The results of our study suggest that if restricted membrane

fluidity does contribute to resistance to complement-mediated lysis it is unlikely to be the only mechanism and that other strategies, e.g. anti-complementary factors (Leid, Suquet & Tanigoshi, 1987) or prevention of complement activation (Pearce, Hall & Sher, 1990), may be involved.

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