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## 先感染外睾吸虫的钉螺其分泌物和血淋巴细胞 对日本血吸虫幼虫的反应\*

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**摘要:**目的 单独感染外睾吸虫的湖北钉螺, 螺体分泌物和血淋巴细胞剧烈增加; 螺体表分泌物含大小不等颗粒及晶状物, 体内分泌物为金黄色或褐色小颗粒, 各颗粒团附近均有极小细胞核。双重感染外睾吸虫和日本血吸虫的钉螺, 体内分泌物和血淋巴细胞等产物同样大量产生; 体内分泌物颗粒及其附近小细胞核、3种血淋巴细胞及副腺细胞, 出现在血吸虫幼虫周围并进入虫体内; 所有血吸虫幼虫结构异常, 停止发育并死亡。阴性钉螺和单独感染日本血吸虫的钉螺, 它们体内分泌物及血淋巴细胞的数量均少; 分泌物颗粒和血淋巴细胞虽有在血吸虫幼虫附近, 但都没有进入虫体内。

**关键词:** 日本血吸虫; 外睾吸虫; 湖北钉螺; 分泌物; 血淋巴细胞

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### Reactions of snail secretions and lymphocytes to *Schistosoma japonicum* larvae in *Oncomelania hupensis* pre-infected with *Exorchis* trematode

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**ABSTRACT:** The objective was to understand the reactions of snail secretions and lymphocytes to the larval *Schistosoma japonicum* in *Oncomelania hupensis* snails pre-infected with *Exorchis* sp. and they were compared with the conditions of negative *O. hupensis* snails, snails singly infected with *Exorchis*, and singly infected with *S. japonicum*. In the snails at 12-105 d after infection with *Exorchis*, snail secretions and lymphocytes increased much. Those situations were also found in the *O. hupensis* snails dual infected with *Exorchis* and *S. japonicum*. Thick secretion with granular and crystal structure on snail body surface and in snail inner tissue were much small secretive granules with some small cell nuclei, lymphocyte with spherical nuclei of 3 varying sizes and accessory gland cells, some of which were found in the bodies of all abnormal *S. japonicum* larvae. In the negative *O. hupensis* and snails singly infected by *S. japonicum* the secretions and lymphocytes were very few. Neither secretive granule nor lymphocytes was found in any bodies of *S. japonicum* larvae in *O. hupensis* snail singly infected by *S. japonicum*.

**KEY WORDS:** larval *Schistosoma japonicum*; *Exorchis* trematode; *Oncomelania hupensis*; secretions; lymphocytes

外睾吸虫 (*Exorchis* spp.) 阳性螺有血淋巴细胞及特殊分泌物大量增生的现象, 经外睾吸虫幼虫先感染的湖北钉螺 (*Oncomelania hupensis*) 能击毙再进入其体内全部日本血吸虫 (*Schistosoma japonicum*) 早期母胞蚴 (1-5、16)。近年, 国外许多学

者报道曼氏血吸虫 (*S. mansoni*) 幼虫与其中间宿主双脐螺 (*Biomphalaria glabrata*) 复杂的相互作用, 涉及螺类宿主血淋巴细胞的生理生化、蛋白酶、免疫应答通路以及基因等诸多情况 (6-15、17)。我们在外睾吸虫和日本血吸虫双重感染的钉螺体中, 观察到螺体内血淋巴细胞、分泌颗粒与其附近小细胞核及副腺细胞存在于异常血吸虫幼虫体内, 而在单独感染血吸虫的钉螺体中无此情况。估计钉螺体内的血淋巴细胞及分泌物中某些物质对入侵的血吸虫幼虫有重要防御作用。为了要对它们深入研究, 我们先对不同条件的钉螺其体内外分泌物和血淋巴细胞

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种类,及它们对血吸虫幼虫反应情况,进行了观察。结果介绍如下。

## 1 材料与方 法

人工感染日本血吸虫的阳性小白鼠和从湖南汉寿西洞庭湖岸边采集的一批湖北钉螺都在实验室内饲养备用。用从实验小白鼠肝脏取得日本血吸虫虫卵孵化毛蚴,每粒钉螺与40个毛蚴接触,钉螺在感染后5~52 d间分别用10%福尔马林溶液固定保存;从湖南汉寿西洞庭湖捕捉的鲢鱼肠管收集外睾吸虫,取其虫卵和少量面粉相拌饲食感染钉螺,感染后16~19 d分别用10%福尔马林溶液固定保存;感染了外睾吸虫的另一些钉螺,分别在感染后21 d将它们再重复感染日本血吸虫,每粒外睾吸虫阳性钉螺接触日本血吸虫毛蚴数分别为20~90条,在感染血吸虫毛蚴后4~82 d间不同时间用10%福尔马林溶液固定保存。上述3组实验钉螺和一些天然阴性钉螺,全部经石蜡连续切片染色制片,显微镜油镜检查各切片的所有断面,比较观察各组钉螺体内外分泌物结构和血淋巴细胞种类及其出现情况,以及它们与螺体内日本血吸虫幼虫接触情况;显微照相储存于电脑。实验钉螺经连续切片观察均无其它吸虫幼虫天然感染。

## 2 结 果

在外睾吸虫阳性钉螺的循环系统和螺体组织中观察到细胞核大小不同3种血淋巴细胞,细胞核均是圆球状,具中等大细胞核的血淋巴细胞数目最多。大、中、小3种血淋巴细胞核的直径分别为:8~8.7  $\mu\text{m}$ 、5.2~5.9  $\mu\text{m}$ 、4.2~4.7  $\mu\text{m}$ 。在螺体组织切片中,见到体表外有粘液状分泌物,体内有金黄色微小颗粒状分泌物,各颗粒团都伴有一个坚实圆形深色极小细胞核(直径1.9~2.6  $\mu\text{m}$ )(图8、9、11)。在螺体内脏团上端侧方的副腺(Accessory gland)部位,有许多大小不同副腺细胞,细胞核直径6.6~10.6  $\mu\text{m}$ 。这些细胞和分泌物颗粒可通过循环管道被输送到身体各处。但是钉螺体内外分泌物结构、血淋巴细胞及副腺细胞的数目,在不同条件的实验钉螺体上表现的情况有所不同。

### 2.1 阴性钉螺分泌物和血淋巴细胞的表现情况

在没有任何吸虫幼虫寄生的阴性正常钉螺,它们体表的分泌物稀薄,全部为粘液状结构,稍厚部位如重叠的云层(图1)。在螺体头足靠边缘部位的组织中,散布若干淡黄色小颗粒状分泌物,颗粒大小和形状不规则,各颗粒团边沿见有一粒深色极小的细胞核(图2)。在螺体内脏团各部位中的循环系统管道非常微细,均不易查见。在消化腺部位组织中只见到数目极少的中等大血淋巴细胞和小血淋巴细胞(图3)。在生殖腺部位的组织中,血淋巴细胞数稍

多一些,可见大、中、小3种血淋巴细胞核(图4)。

2.2 单独感染日本血吸虫的钉螺其分泌物和血淋巴细胞的表现情况 单独感染日本血吸虫的钉螺,其体外稀薄粘液状分泌物、体内为数不多血淋巴细胞和颗粒状分泌物等情况,均和阴性钉螺无大差别(图5-8)。感染后5~8 d的钉螺(图5-6),在血吸虫早期幼虫附近的螺组织中,有少量金黄色小颗粒状分泌物;感染后10~52 d的钉螺(图7-8),在血吸虫母胞蚴附近螺组织中,有一些血淋巴细胞及较多颗粒分泌物,在有的胞蚴体壁上有许多细小黑色颗粒(图7),它们都没有进入胞蚴体内(图7-8)。血吸虫幼虫的发育没有受到影响。

2.3 单独感染外睾吸虫的钉螺其分泌物和血淋巴细胞的表现情况 单独感染外睾吸虫的钉螺已实验确认,96%(157/163)都有体表分泌物和血淋巴细胞增加现象(图9-12)。在感染后16~19 d的钉螺体组织中很容易找到3种血淋巴细胞,中大血淋巴细胞的数量较多;螺体组织中着许多金黄色细小颗粒状分泌物,它们散布无论稀疏或浓密都伴随有尚未知晓的极小细胞核(图9、11)。螺体整个体表分泌物增厚,在分泌物粘液膜上密布着大小及形状不同的各种颗粒,并有许多晶体结构的物质(图10)。在螺体内脏团的消化腺区域,循环系统所有血淋巴管都明显扩大。管内含有各种血淋巴细胞、螺体分泌物小颗粒及与其在一起的小细胞核(图11),副腺细胞在管内亦清晰可见(图12)。

2.4 外睾吸虫和日本血吸虫双重感染的钉螺其分泌物和血淋巴细胞表现情况 外睾吸虫和日本血吸虫两者间隔21 d先后感染的钉螺,后感染的血吸虫虫体刚进入螺体,立即出现异常。虫体附近出现许多含极小细胞核的分泌物小颗粒、各种血淋巴细胞和副腺细胞,并进入4 d的异常虫体体内(图13-14)。异常虫体内常有1~3个未知的大红色球团(图13),它们表面密布暗淡小颗粒。这红团块可持续存在数十天。血吸虫感染后16~21 d(图15-16)和38~39 d(图17-18)的钉螺组织中许多含极小细胞核的小颗粒分泌物、各种血淋巴细胞和副腺细胞,亦见于所有异常血吸虫附近及其体内。感染后82 d(图19-20)的异常血吸虫幼虫在螺体不同部位,虫体生长情况不同,但螺体的反应和后果是相同的。如在螺体围心腔附近的一个大的异常母胞蚴(图19),螺体各种血淋巴细胞和含极小细胞核的咖啡色颗粒分泌物等大量包围并侵入虫体,它中间的一团已近解体;在螺体外套膜上的小异常母胞蚴(图20),螺组织中血淋巴细胞和含极小细胞核的金黄色

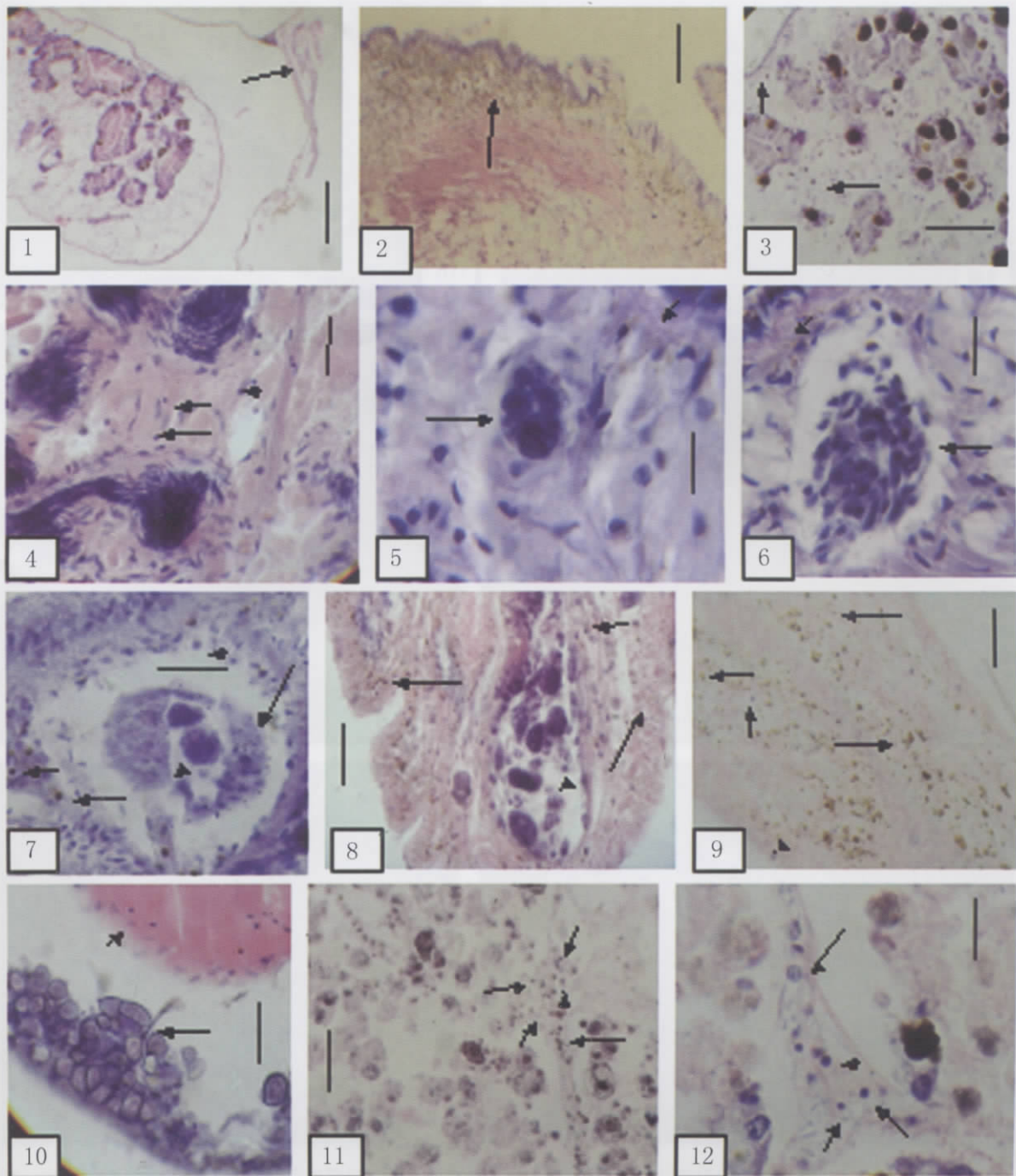
颗粒分泌物数量不多,同样也有少量侵入到虫体内,虫体同样受影响。

### 3 讨论

血吸虫病是没有疫苗可以控制的疾病,世界上70多个国家约2亿人受威胁。其传播媒介是螺类,血吸虫和螺类宿主的相互关系(schistosoma-snail host interaction)是预防此病害需要了解的知识。国外对曼氏血吸虫(*Schistosoma mansoni*)幼虫与媒

介双脐螺(*Biomphalaria glabrata*)的相互关系,进行了大量研究工作<sup>[6]</sup>。螺体有保护自己不受病原感染的内在防御系统(internal defense system),其免疫反应涉及血液中的血淋巴细胞、蛋白质组学、基因组学及多方面生理生化问题。血浆携带酶(plasma-borne-enzymes)、调理素(opsonins)、血淋巴细胞产生Cu/Zn超氧化歧化酶、过氧化氢(H<sub>2</sub>O<sub>2</sub>)、具反应性氧种类(reactive oxygen species)产物、氮氧化(nitric oxide)等的参与、及糖类特异性和某些蛋白质酶

先感染外睾吸虫的钉螺其分泌物和血淋巴细胞对日本血吸虫幼虫的反应





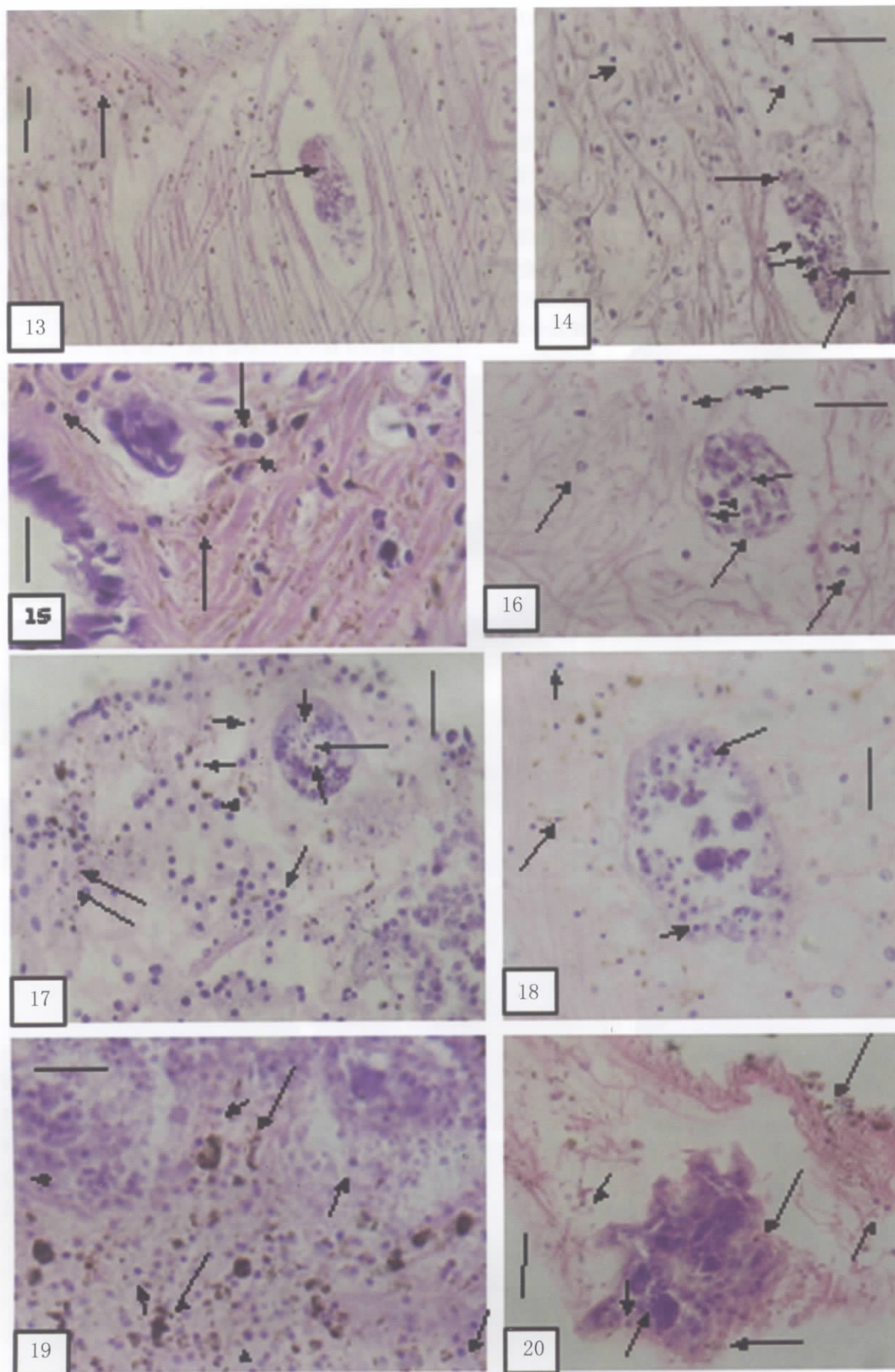


图 1-4 阴性湖北钉螺切片,示螺体表分泌物、体内金黄色颗粒状分泌物和血淋巴细胞

Figs. 1-4 Sections of negative *Oh* snails, showing snails body surface secretion, secretive granules in body and lymphocytes

图 5-8 单独感染日本血吸虫幼虫的湖北钉螺切片,示螺体分泌物和血淋巴细胞,及其对血吸虫幼虫的反应

Figs. 5-8 Sections of *Oh* snails singly infected by *Sj*, showing snail secretions, lymphocytes and their reactions to *Sj* larvae

图 9-12 单独感染外睾吸虫的湖北钉螺切片,示螺体分泌物及血淋巴细胞

Figs. 9-12 Sections of *Oh* snails singly infected with *Ex* trematode, showing the snail's secretion and lymphocytes

图 13-20 外睾吸虫与血吸虫间隔 21 天双重感染湖北钉螺切片,示螺体分泌物及血淋巴细胞等对血吸虫幼虫的反应

Figs. 13-20 Sections of *Oh* snails dually infected by *Ex* sp. and *Sj* at 21d intervals, showing the snail secretions, lymphocytes and accessory gland cells, and their reactions to the post-infected *Sj* larvae

图 1 在阴性钉螺体表的少而稀薄的分泌物(scale bar = 0.15mm)

Fig.1 Few and thin secretion on body surface of negative *Oh* snail

图 2 阴性钉螺体内金黄色分泌物颗粒(scale bar = 0.06mm)

Fig.2 Golden yellow secretive granules inner body of negative *Oh* snail

图 3 少数中大和小的血淋巴细胞在阴性钉螺消化腺部位(scale bar = 0.08mm)

Fig.3 Some middle and smallest lymphocytes in the digestive gland area of negative *Oh* snail

图 4 少数大、中、小的血淋巴细胞在阴性钉螺精巢部位(scale bar = 0.049mm)

Fig.4 Some large, middle and smallest lymphocyte in testes area of negative *Oh* snail

图 5 螺体一些金黄色分泌物颗粒在 5d 血吸虫幼虫附近(scale bar = 0.023mm)

Fig.5 Some yellow golden secretive granules of snail were nearby the 5d old early larva of *Sj*

图 6 螺体一些金黄色分泌物颗粒在 8d 血吸虫幼虫附近(scale bar = 0.023mm)

Fig.6 Some yellow golden secretive granules of snail were nearby the 8d old *Sj* larva

图 7 螺体少数血淋巴细胞在 10d 血吸虫母胞蚴附近,分泌物小颗粒在母胞蚴体表上,不见于血吸虫体内(scale bar = 0.049mm)

Fig.7 Some large, middle and smallest lymphocytes in snail were nearby the 10d old *Sj* mother sporocyst, small black secretive granules on the mother sporocyst surface, which have not been in sporocyst coeloma

图 8 螺体少数血淋巴细胞和金黄色分泌物颗粒在 52d 血吸虫母胞蚴附近,它们不见于血吸虫体内(scale bar = 0.054mm)

Fig.8 Some lymphocytes, many golden yellow secretive granules with small cell nucleus in snail were nearby 52d old *Sj* mother sporocyst, which have not been in the sporocyst coeloma

图 9 16d 外睾吸虫阳性钉螺体内许多大、中、小血淋巴细胞及具小细胞核金黄色分泌物颗粒(scale bar=0.065mm)

Fig.9 Many large, middle and small lymphocytes and much golden yellow secretive granules with small cell nucleus in the body of *Oh* snail infected with *Ex* sp. for 16d

图 10 晶体结构分泌物在 22d 外睾吸虫阳性钉螺体表(scale bar=0.03mm)

Fig.10 Secretion with crystal structure on the body surface of *Oh* snail infected by *Ex* sp. for 22d

图 11 许多血淋巴细胞及分泌物颗粒在 19 d 外睾吸虫阳性钉螺消化腺区扩大的血淋巴管中(scale bar = 0.099mm)

Fig.11 Many large, middle and small lymphocytes, and secretive granules with small cell nucleus in the enlarged lymphatic ducts at digestive gland area of *Oh* snail infected by *Ex* sp. for 19d

图 12 大、中、小血淋巴细胞和副腺细胞在 19 天外睾吸虫阳性钉螺消化腺区扩大淋巴管中(scale bar=0.039mm)

Fig.12 Large, middle and small lymphocytes, and accessory gland cells in the enlarged lymphatic duct at digestive area of *Oh* snail infected by *Ex* sp. for 19d

图 13 具小细胞核的金黄色分泌物颗粒在螺体组织中及红色球体在 4 d 异常血吸虫幼虫体内(scale bar = 0.041mm)

Fig.13 Much golden yellow secretive granules with small cell nucleus in snail tissue and a large red sphere in the body of 4d old abnormal *Sj* larva

图 14 血淋巴细胞及具小细胞核的金黄色分泌物颗粒在螺体组织中及 4 d 异常血吸虫幼虫体内(scale bar = 0.06mm)

Fig.14 Many large, middle and small lymphocytes and much golden yellow secretive granules with small cell nuclei in snail tissue and in the body of 4d old abnormal *Sj* larva

图 15 具小细胞核的分泌物颗粒和血淋巴细胞在螺体组织及 16 d 的异常血吸虫母胞蚴附近(scale bar=0.029mm)

Fig.15 Much yellow golden secretive granules with small cell nucleus and many large, middle and small lymphocytes in the snail tissue and nearby the body of 16d old abnormal mother sporocyst of *Sj*

图 16 大、中、小血淋巴细胞及副腺细胞在螺体组织及 21 d 异常血吸虫母胞蚴体内(scale bar = 0.046mm)

Fig.16 Large, middle and small lymphocytes and the accessory gland cells in the snail tissue and the body of 21d old abnormal mother sporocyst of *Sj*

图 17 血淋巴细胞及具小细胞核的黑色分泌物颗粒在螺体组织及 38 d 常血吸虫母胞蚴体内(scale bar = 0.051mm)

Fig.17 Many large, middle and small lymphocytes and black secretive granules with small cell nucleus in the snail tissue and in the body of 38d old abnormal mother sporocyst of *Sj*

图 18 血淋巴细胞和具小细胞核分泌物颗粒在螺体组织及 39 d 异常血吸虫母胞蚴体内(scale bar = 0.046mm)

Fig. 18 Some lymphocytes and golden yellow secretive granules with small cell nucleus in the snail tissue and in the body of 39d old abnormal mother sporocyst of *Sj*

图 19 血淋巴细胞及具小细胞核的分泌物在螺体围心腔组织及 82 d 异常血吸虫母胞蚴体内 (scale bar = 0.040mm)

Fig. 19 Many large, middle and small lymphocytes, and much black secretion with small cell nucleus in the pericardial tissue of snail and in the body of 82d old abnormal mother sporocyst of *Sj*

图 20 血淋巴细胞和具小细胞核的分泌物颗粒在螺体外套膜组织中及 82 d 异常血吸虫母胞蚴体内 (scale bar = 0.045mm)

Fig. 20 Some lymphocytes and golden yellow secretive granules with small cell nucleus in the tissue of snail mantle membrane and in the body of 82d old abnormal mother sporocyst of *Sj*

情况等,在螺宿主抵抗血吸虫早期幼虫和胞蚴都发挥作用<sup>[8-12,17]</sup>。发现在双脐螺中对曼氏血吸虫具抗性的螺(*S. mansoni* resistant snails)会产生更多可杀死血吸虫胞蚴的过氧化氢( $H_2O_2$ )比过对血吸虫敏感的螺(*S. mansoni* susceptible snails)<sup>[7]</sup>。有的学者对双脐螺的血淋巴细胞表面受体(surface receptor)、基因、相关的免疫应答通路等都开展深入工作<sup>[13-15,17]</sup>。

有关日本血吸虫和媒介钉螺的相互作用问题欠缺科学资料。在先感染外睾吸虫的钉螺,再侵入螺体的血吸虫幼虫立即被击毁,钉螺有大量血淋巴细胞和各种分泌物产生<sup>[1-2,4,16]</sup>。本文实验证明不同条件的钉螺产生分泌物和血淋巴细胞及其对血吸虫的反应情况有很大差异,它们的血淋巴细胞和分泌物对血吸虫幼虫产生不同防御机理,是如何起变化,有待深入研究。

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