Schistosoma haematobium e cancro da bexiga: a verdade escondida

Mónica Botelho

9/4/2013
What is schistosomiasis?
BEWARE

BILHARZIA

ERECTED BY ASSOCIATION FOR BILHARZIA CONTROL
P.O. BOX 2171 BULAWAYO
Schistosomiasis: facts and figures

- Human schistosomes currently infect more than 200 million people in 76 countries worldwide in the endemic areas of Africa, the Caribbean, Central America, South America, East Asia, and the Middle East.
Global distribution of Schistosomiasis

- **Senegal**: An epidemic of schistosomiasis along the Senegal river basin caused by water-resource development schemes continues unabated.
- **Egypt**: Praziquantel chemotherapy coupled to a vigorous media campaign has resulted in a significant decrease in the morbidity and prevalence of schistosomiasis infection.
- **Iran, Morocco, and Saudi Arabia**: Schistosomiasis control has been successful in those areas with elimination of the infection contemplated.
- **China**: Schistosoma continues to be a major public health problem in the lake and marshy regions despite successful control in other endemic areas.
- **Lao People's Democratic Republic**: Schistosoma mekongi control has been successful around Khong Island with prevalence reduced from 42% to < 2%.

**Djibouti and Somalia**: Displacement of people by war and instability has introduced intestinal schistosomiasis to these countries.

- **North-east Brazil**: Urban schistosomiasis now present in and around many major cities.
- **Ghana**: Intestinal schistosomiasis has increased due to the construction of the Akosombo Dam and other much smaller dams.
- **sub-Saharan Africa**: More than 85% of the estimated 200 million people globally with schistosomiasis and the majority of patients with severe disease live on this continent.
- **Indonesia**: Schistosomiasis has been controlled in the Lindu region of Sulawesi such that the prevalence of infection is lower than 2%.
• Is there a role for *S. haematobium* in bladder cancer?
S. haematobium and bladder cancer

- A causal association between the parasite and bladder cancer was postulated in 1911 by Fergusson, but so far proof of this association has remained elusive.
S. haematobium and bladder cancer

Squamous cell carcinoma of the urinary bladder has been associated with *Schistosoma haematobium* infection in many parts of Africa.

A parasite-tumor linkage is further suggested by the predominance of squamous cell (as opposed to transitional cell) morphology of bladder carcinomas seen in *S. haematobium*-endemic areas.
• What about host endocrine system?
Schistosomiasis and host hormones

It has been shown that schistosomes synthesizes steroid hormones (Nirde et al, FEBS Letters, 1983).

Schistosomes produce hormone-like signals (Mendonça et al, Parasitology Today, 2000).

Existence of receptors able to bind the molecules of estradiol (Barrabes, Ann Parasitol Hum Comp, 1986; Mendonça et al, Parasitology Today, 2000).
Recent experimental evidence suggests that schistosomes can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment, growth and reproduction (Escobedo et al, Trends in Parasitology, 2005).
Aims

- A) To study the mechanisms underlying the association between *S. haematobium* and SCC of the bladder
- B) To understand how host endocrine system can favor the establishment of schistosomes
S. haematobium and bladder cancer
*Schistosoma haematobium* immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs.
Methodological strategy
Methodological strategy

Schistosomiasis

Cercariae

Snails

Adult worms

Miracidia

Eggs

Experimental infection

Or

Mouse

Skin

Schistosomulum

Male worm

Female worm

Mating

Eggs
*Schistosoma haematobium* immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs

Botelho *et al.* Virulence 2010
*S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro*. 
Methodological Strategy

S. haematobium total antigen (Sh)
Methodological Strategy

Chinese Hamster Ovary (CHO) cells

Sh-treatment 48 h
Methodological Strategy

Proliferation MTT assay

Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells.
Methodological Strategy

DNA content  Flow cytometry

Cell Cycle

- **G₀**: Quiescence (variable)
- **G₁**: Synthesis of components required for DNA synthesis (40%)
- **S**: DNA synthesis (39%)
- **G₂**: Preparation for mitosis (19%)
- **M**: Mitosis and cell division (2%)

Graph showing DNA content distribution with peaks at **G₀/G₁**, **S**, and **G₂/M**.
The western blot is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.
*S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro.*
Methodological Strategy

Apoptosis TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.
Methodological Strategy

1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37 C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and stay in the upper chamber.
*S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro*

Botelho *et al.*, Int J Parsitol 2009
S. haematobium total antigen induces tumorigenesis
Methodological Strategy

Chinese Hamster Ovary (CHO) cells

Control CHO cells

Sh-treated CHO cells

Sh-treatment 48 hours

No Tumour

Tumour-Bearing Nude Mouse

Nude mice

2 weeks
S. haematobium total antigen induces tumorigenesis

Schistosoma haematobium total antigen induces dysplasia and KRAS gene mutations in CD-1 mice normal urothelium.

Pathogenic pathways of rat and mice urinary bladder carcinogenesis.
Methodological Strategy

Transurethral Catheterization

Urothelial Denudation

Sh-treatment /PBS 1 hour

In situ fixation 10% PPA

20 weeks
40 weeks
Methodological Strategy
Schistosoma haematobium total antigen induces dysplasia and KRAS gene mutations in CD-1 mice normal urothelium

Botelho et al. (Submitted)
Schistosomiasis and host hormones
Shistosoma haematobium produces an estradiol-related molecule

Estradiol (E2)
**Shistosoma haematobium** produces an estradiol-related molecule

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<th>Age (years)</th>
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<table>
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<th>Antigenic preparations</th>
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<tr>
<td><em>S. haematobium</em></td>
<td>14,84±0,14</td>
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<td><em>S. mansoni</em></td>
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<td><em>F. haepatica</em></td>
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<td><em>H₂Od</em></td>
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<td>10 nM E2</td>
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Botelho et al. Exp Parasitol 2009
*S. haematobium* produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity.
Methodological Strategy

Gene expression Real-Time PCR
Methodological Strategy

Transfection pERE-Luc

[Diagram of pGL3-Basic Vector with EcoRI, f1 ori, ori, Amp', and luc+ indicated, along with restriction enzyme sites and their corresponding restriction sites: KpnI, SalI, SacI, MluI, NheI, Smal, XhoI, BglII, BamHI, NcoI, NsiI, XbaI, Sall, and Hpal.]

Estrogen Responsive Element (ERE)
Methodological Strategy

Mass spectrometry LC-ESI-MS
**S. haematobium** produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity.

**Gene Expression**

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Fold increase

- ER alpha
- ER beta

**pERE-Luc transfection**

- Control
- E2
- ICI
- E2+ICI
- Sh
- Sh+E2
- Sh+ICI
- Sh+E2+ICI

Luciferase activity
*S. haematobium* produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity.
S. haematobium produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity.
Schistosomiasis, bladder cancer and host hormones
*Schistosoma haematobium* total antigen down-regulates ER alpha and ER beta in HCV29 normal urothelial cells and down-regulates ER expression in the bladders of CD1 mice.
Conclusions

*S. haematobium* and bladder cancer

*S. haematobium* total antigen induced increased proliferation, decreased apoptosis, and induced migration and invasion of normal epithelial cells in culture. In addition, the parasite extract has also the potential to induce tumour development, assessed by the use of a nude mice xenograft model.

According to our findings, *S. haematobium* total antigen in CD-1 mice normal bladders after intravesical instillation of the parasite antigen, induced alterations in the urothelium of these animals consistent with dysplasia and inflammation. In these animals, we also found that the parasite extract of *S. haematobium* has carcinogenic ability possibly through oncogenic mutation of *KRAS* gene.
**Conclusions**

*S. haematobium* and hormones

*S. haematobium* total antigen expresses estradiol-related molecules that down regulate Estrogen Receptor alpha and beta in estrogen responsive cells. These estrogens are also present in the sera of *Schistosoma*-infected individuals, and they have the ability to repress Estrogen Receptor transcriptional activity.

The estrogenic molecules present in *S. haematobium* extracts could have a carcinogenic effect possibly through estrogen adduct-mediated pathway and could further explain the link between this parasite and squamous cell carcinoma of the bladder.
Therefore, these results may open potential new strategies for cancer diagnosis by using these estrogens as biomarkers in schistosomiasis-associated bladder cancer.
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Maria José Oliveira
António Carlos Ferreira
Joana Gomes

UTAD
Professor Paula Oliveira
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Ligia Lourenço

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Dr. Gabriela Martins
Isabel Veiga
Madalena Crespo
Carlos Palmeira

Dr. Luis Araújo

IPORTO Instituto Português de Oncologia do Porto FGPE
Thank you for your attention
Origin: Adult schistosomes in blood vessels around small intestine

Stimulus: Eggs laid by female are carried in blood vessels and trapped in liver

Response: Hypersensitivity to antigens of larva inside egg cause formation of granuloma. Liver sinusoids become blocked, impeding blood flow

Pathology: Fibrosis of liver
  - Raised portal pressure
  - Perihepatic shunting of blood
  - Hepatomegaly
  - Splenomegaly
  - Formation of varices
Histopathological data in the livers of infected animals.

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<th>Group</th>
<th>Hamster</th>
<th>P</th>
<th>PH</th>
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</table>

P, *S. haematobium* in histological section; PH, fibrous perihepatitis; F, lymphoid follicles; LPI, infiltration of lymphocytes and plasma cells; BP, bilharzial pigment; E, infiltration of eosinophils; MT, Masson Trichrome stain; R, Reticulin stain; -, absent; +, mild; ++, moderate; ++++, severe.
Histological findings of livers of infected animals.
Histological findings of livers of infected animals.
Schistosoma haematobium total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells

Monica Botelho, António Carlos Ferreira, Maria José Oliveira, José Carlos Machado and José Manuel Correia da Costa

(International Journal for Parasitology, 2009)
Methodological Strategy

S. haematobium total antigen (Sh)
Methodological Strategy

Chinese Hamster Ovary (CHO) cells

Sh-treatment 48 h
S. haematobium total antigen induces alterations in the morphology of treated cells

Morphological differences of CHO cells in culture. Control in the left panels and Sh-treated cells in right panels.
Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. [1]
*S. haematobium* total antigen, but not *S. mansoni* total antigen, increased the proliferation of epithelial cells in vitro.

**Cell Proliferation** *S. haematobium* vs. *S. mansoni*

Comparison of proliferation between Sh and Sm treated cells (*p*<0.01; Sh vs. Sm).
Methodological Strategy

DNA content  Flow cytometry

Cell Cycle

- $G_2$
- Preparation for mitosis (13%)
- $M$
- Mitosis and cell division (2%)
- $G_0$
- Quiescence (variable)
- $G_1$
- Synthesis of components required for DNA synthesis (40%)
- $S$
- DNA synthesis (39%)
*S. haematobium* total antigen altered the cell cycle distribution decreasing G0/G1 and increasing S- and G2/M-phase.

<table>
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<td>S</td>
<td>G2/M</td>
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<td>78.07</td>
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Channels
The western blot is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.
Methodological Strategy

Apoptosis

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.
**S. haematobium** total antigen decreased the apoptosis of epithelial cells *in vitro*

Apoptosis of Sh treated cells analysed by TUNEL. The experiments were done in triplicate (\(*p<0.01; \)control vs. 50 microg/ml).
Up-regulation of the anti-apoptotic protein Bcl-2, in vitro, by *S. haematobium* total antigen

Histogram of CHO cells expressing BCL-2 by FACS analysis. Control left panel and Sh treated cells right panel.

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Induced migration of epithelial cells *in vitro* by *S. haematobium* total antigen

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Wound closure assay
Methodological Strategy

1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37°C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and stay in the upper chamber.
Induced invasion of epithelial cells *in vitro* by *S. haematobium* total antigen

Effect of Sh on cell invasion (p<0.01; control vs. Sh treated cells).

Control | Sh-treated
Tumorigenic effect of *Schistosoma haematobium* total antigen in normal mammalian cells


(Int. J. Exp. Path., 2009)
Methodological Strategy

Experimental infection

Or

Mouse

Skin

Schistosomulum

Male worm

Female worm

Mating

Eggs

S. haematobium total antigen (Sh)
Methodological Strategy

Chinese Hamster Ovary (CHO) cells → Sh-treatment 48 hours → No Tumour

Control CHO cells

Sh-treated CHO cells → 2 weeks → Tumour-Bearing Nude Mouse

Nude mice
Sh allows the vigorous growth of CHO xenografts in nude mice.
## Immunohistochemistry and histochemical staining

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumour</th>
<th>Cytokeratin</th>
<th>Vimentin</th>
<th>AgNORs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(Sh)</td>
<td>Sarcoma</td>
<td>-</td>
<td>+</td>
<td>1,97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,09</td>
</tr>
<tr>
<td>1(Sh)</td>
<td>Sarcoma</td>
<td>-</td>
<td>+</td>
<td>1,94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,06</td>
</tr>
<tr>
<td>1(Sh)</td>
<td>Sarcoma</td>
<td>-</td>
<td>+</td>
<td>1,72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0,9</td>
</tr>
<tr>
<td>1(Sh)</td>
<td>Sarcoma</td>
<td>-</td>
<td>+</td>
<td>1,88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,09</td>
</tr>
<tr>
<td>1(Sh)</td>
<td>Sarcoma</td>
<td>-</td>
<td>+</td>
<td>1,86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,12</td>
</tr>
<tr>
<td>2(Ctrl)</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2(Ctrl)</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2(Ctrl)</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
Histological findings of the tumour mass produced by inoculation of CHO cells exposed to Sh
Induced dysplasia and inflammation of *Schistosoma haematobium* total antigen on normal urothelium of CD-1 mice

Botelho M, Oliveira PA, Lopes C, Correia da Costa JM and Machado JC

(Urologic Oncology: Seminars and Original Investigations, 2009)
Methodological Strategy

Experimental infection

$\text{S. haematobium}$ total antigen (Sh)
Methodological Strategy

1. Transurethral Catheterization
2. Urothelial Denudation
3. In situ fixation 10% PPA
4. Sh-treatment /PBS 1 hour
5. 20 weeks
   40 weeks
Methodological Strategy
Summary of the histopathological data in CD-1 mice urothelium exposed to Sh

<table>
<thead>
<tr>
<th>Group</th>
<th>Umbrella cells</th>
<th>Inflammation</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>(5/10) 50%</td>
<td>(9/10) 90%</td>
<td>(3/10) 30%</td>
</tr>
<tr>
<td>Control</td>
<td>(9/10) 80%</td>
<td>(2/10) 20%</td>
<td>(0/10) 0%</td>
</tr>
<tr>
<td>40 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>(0/10) 0%</td>
<td>10/10 (100%)</td>
<td>(7/10) 70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P=0.001</td>
</tr>
<tr>
<td>Control</td>
<td>(10/10) 100%</td>
<td>3/10 (30%)</td>
<td>(0/10) 0%</td>
</tr>
</tbody>
</table>
Histological findings of CD-1 mice urothelium exposed to Sh
Carcinogenic ability of *S. haematobium* through oncogenic mutation of KRAS gene

Mónica Catarina Botelho, José Carlos Machado and José Manuel Correia da Costa
(Virulence, 2010)
KRAS exon 2 sequencing results
• Is there a role for *S. haematobium* in bladder cancer?
• Is there a role for *S. haematobium* in bladder cancer?

• Yes!
• Is there a role for *S. haematobium* in bladder cancer?

• Yes!

Pathogenic pathways of rat and mice urinary bladder carcinogenesis.
Take home message I

- *S. haematobium* total antigen induced increased proliferation of normal epithelial cells in culture.

- *S. haematobium* alters the cell cycle of treated cells as demonstrated by increased S-phase and down-regulation of the CDKI p27 expression of normal epithelial cells in culture.

- p27 causes cell cycle arrest in G1 phase and abrogating its function results in uninhibited cell proliferation and tumorigenesis.
• *S. haematobium* dramatically decreased the apoptosis of epithelial cells by up-regulating Bcl-2 protein.
• Treated cells with *S. haematobium* total antigen had increased migration than control cells.
• *S. haematobium* induced the invasion of treated cells 5 times more than control cells.
Take home message III

- *S. haematobium* total antigen has the potential to induce tumour development, assessed by the use of a nude mice xenograft model.

- *S. haematobium* total antigen in CD-1 mice normal bladders after intravesical administration of the parasite antigen, induced alterations in the urothelium of these animals consistent with dysplasia and inflammation.

- The parasite extract of *S. haematobium* has carcinogenic ability possibly through oncogenic mutation of KRAS gene.
Credits

- CIBP-INS A Porto
  - José Manuel Costa
  - Paulo Vieira
  - Lurdes Delgado

- IPATIMUP
  - José Carlos Machado
  - Maria José Oliveira
  - António Carlos Ferreira
  - Fatima Gartner
  - Joana Gomes

- FMUP
  - Raquel Soares

- UTAD
  - Paula Oliveira

- ICBAS
  - Carlos Lopes

- IPO Porto
  - Gabriela Martins
  - Carlos Palmeira
  - Manuel Teixeira
  - Isabel Veiga
Specific Aim

- To study schistosoma-associated SCC of the bladder through cancer pathways using *in vitro* and *in vivo* approaches.
Ongoing work

- Animal studies with experimental infections

Pathogenic pathways of rat and mice urinary bladder carcinogenesis.
$S. \text{haematobium}$ and host hormones

- Male hypogonadism is a well known consequence of schistosomiasis (Saad, J Egypt Soc Parasitol, 1999).
- It has been shown that schistosomes synthesizes steroid hormones (Nirde et al, FEBS Letters, 1983).
- Schistosomes produce hormone-like signals (Mendonça et al, Parasitology Today, 2000).
- Existence of receptors able to bind the molecules of estradiol (Barrabes, Ann Parasitol Hum Comp, 1986; Mendonça et al, Parasitology Today, 2000).
**S. haematobium** and host hormones

- Recent experimental evidence suggests that schistosomes can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment, growth and reproduction (Escobedo et al, Trends in Parasitology, 2005).
**Key:**

ER = estrogen receptor,  ERE = estrogen response element,  AF = activation function,  RNA POL II = RNA polymerase II
**S. haematobium** infected patients have increased serum levels of estradiol

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>E2</th>
<th>Range</th>
<th>Testosterone</th>
<th>Range</th>
<th>LH</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4</td>
<td>62,8</td>
<td>0-22</td>
<td>&lt;15,0</td>
<td>2-10</td>
<td>0,114</td>
<td>&lt;2,5</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>30,8</td>
<td>0-25</td>
<td>77,5</td>
<td>5-500</td>
<td>1,79</td>
<td>0,2-8,0</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>79,8</td>
<td>0-25</td>
<td>363</td>
<td>5-500</td>
<td>1,89</td>
<td>0,2-8,0</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>45,7</td>
<td>0-25</td>
<td>724</td>
<td>&gt;200</td>
<td>5,89</td>
<td>1,4-7,7</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>31,9</td>
<td>0-25</td>
<td>535</td>
<td>&gt;200</td>
<td>7,65</td>
<td>1,4-7,7</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>68,3</td>
<td>&lt;56,0</td>
<td>982</td>
<td>262-1593</td>
<td>2,87</td>
<td>1,4-7,7</td>
</tr>
</tbody>
</table>
**S. haematobium** and **S. mansoni** express an estradiol analogue but not **Fasciola hepatica**

<table>
<thead>
<tr>
<th>Antigenic preparations</th>
<th>E2 (pg/ml)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haematobium</em></td>
<td>14.84</td>
<td>0.14</td>
</tr>
<tr>
<td><em>S. mansoni</em></td>
<td>12.63</td>
<td>0.27</td>
</tr>
<tr>
<td><em>F. haepatica</em></td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>H₂Od</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>10 nM E2</td>
<td>1632.99</td>
<td>2.55</td>
</tr>
</tbody>
</table>
*S. haematobium* total antigen decreases estradiol production of CHO cells *in vitro*

Estradiol production in culture. The experiments were done in triplicate (p<0.01; control vs. 50 microg/ml).
ICI 182,780

Estradiol (E2)
**ESTRADIOL**

- Receptor dimerisation
- AF1 + AF2 ACTIVE
- Nuclear localisation of fully active ER to ERE

**FULLY ACTIVATED TRANSCRIPTION**

- Coactivator
- RNA POL II
- AF1 and AF2 recruit coactivators

---

**‘FASLODEX’**

- Accelerated receptor degradation
- Attenuated dimerisation
- AF1 + AF2 INACTIVE
- Reduced nuclear localisation of inactive ER to ERE

**NO TRANSCRIPTION**

- RNA POL II
- ERE
- No coactivator recruitment

---

**Key:**

ER = estrogen receptor,  ERE = estrogen response element,  AF = activation function,  RNA POL II = RNA polymerase II
**S. haematobium** total antigen is an antagonist of estradiol

Estradiol production in supernatant of treated cells. Data represent means ± SEM from 3 independent experiments (* p<0.05 control vs. Sh).
S. haematobium total antigen down-regulates ER beta

RT-PCR for ER alpha and ER beta in CHO cells.
Estrogen Responsive Element (ERE)
Inactivation of Estrogen Receptor signaling pathway by *S. haematobium* total antigen

Role of Sh on inactivation of Estrogen Responsive Element (ERE) assessed by Luciferase Assay. Incubation with Sh resulted in a statistically significant decrease in luciferase activity compared to control (*, P <0.05).
Egg with miracidium

Calcified egg
Hormones

Schistosomula

Adult worms in:
- blood vessel around rectum (mansoni)
- bladder (haematobium)
- mesenteric veins and pulmonary arteries (japonicum)

Egg production (10-30 per day)

Cercaria (500 per year per snail)

Sporocysts (several per miracidium)

Miracidium in water
Hormones
Hormones
Hormones
Geographic distribution of 
Schistosoma haematobium
- Eggs are deposited in water in faeces or urine.
- Adult worms produce eggs that lodge in tissues, causing damage to lungs, liver, spleen, intestine and urinary tract.
- Eggs of *Schistosoma mansoni* and *S. haematobium*.
- Miracidium hatches from egg.
- Miracidium penetrates tissues of snail.
- Free-swimming cercariae penetrate skin.
- Larval worms mature to adults in deep blood vessels (after about 6 weeks).
- After asexual reproduction, thousands of cercariae emerge.
Schistosoma Haematobium

Le Couple

Mâle

Femelle

Hôte intermédiaire, la Bulline

Cercariae

Oeuf
“Welcome to the fabulous world of parasites”

Mónica Botelho
06/07/07
• What is a parasite?
• **Parasitism**, wherein one organism, usually physically smaller of the two (the *parasite*) benefits and the other (the *host*) is harmed.
Parasitism is one version of symbiosis ("living together"), a phenomenon in which two organisms which are phylogenetically unrelated co-exist over a prolonged period of time, usually the lifetime of one of the individuals.
List of parasitic organisms

- **Protists (Protozoa)**
  - *Giardia lamblia* (the most common intestinal protozoan in the United States)
  - *Naegleria fowleri* (facultative parasite causing amoebic meningitis)
  - *Entamoeba histolytica* (causes Amebiasis, common in developing countries)
  - *Trypanosoma* (sleeping sickness or Chagas disease)
  - *Plasmodium* (malaria)
  - *Toxoplasma* (toxoplasmosis)

- **Helminths**
  - *Ascariasis* (roundworms)
  - *Cestoda* (tapeworms) including: *Taenia saginata* (human beef tapeworm), *Taenia solium* (human pork tapeworm)
  - *Enterobius vermicularias* (pinworm)
  - *Filariasis*
  - *Schistosomiasis* or *Bilharziosis*
• *Schistosoma haematobium*
# Tumorigenic effect of *S. haematobium* total antigen on normal epithelial cells

<table>
<thead>
<tr>
<th>Nude Mice</th>
<th>Dia 15 (mm³)</th>
<th>Dia 20 (mm³)</th>
<th>Dia 30 (mm³)</th>
<th>Dia 90 (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh1</td>
<td>0</td>
<td>400</td>
<td>4416</td>
<td>Sacrif.</td>
</tr>
<tr>
<td>Sh2</td>
<td>0,125</td>
<td>6375</td>
<td>Sacrif.</td>
<td>-</td>
</tr>
<tr>
<td>Sh3</td>
<td>0,125</td>
<td>1584</td>
<td>Sacrif.</td>
<td>-</td>
</tr>
<tr>
<td>Sh4</td>
<td>2808</td>
<td>7200</td>
<td>Sacrif.</td>
<td>-</td>
</tr>
<tr>
<td>Sh5</td>
<td>0</td>
<td>0</td>
<td>1870</td>
<td>Sacrif.</td>
</tr>
<tr>
<td>C1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0,125</td>
</tr>
<tr>
<td>C2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Tumorigenic effect of *S. haematobium* total antigen on normal epithelial cells

- **Histopathology**
  - Inflammatory tumors
  - Lymphoid tissue
Take home message II

• *S. haematobium* infected patients have increased serum levels of estradiol, most likely *Schistosoma* derived.

• *S. haematobium* total antigen expresses an analog of estradiol.

• *S. haematobium* total antigen decreased estradiol levels in culture, acting as an estradiol antagonist.

• *S. haematobium* total antigen down-regulates Estrogen Receptor beta.

• In agreement with these data, we found that *S. haematobium* total antigen was able to inactivate ER transcription through the use of a pERE-Luc.
Granulomatous-like immune reaction and hepatic fibrosis induced by *Schistosoma haematobium* immature worms
