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PRECLINICAL STUDIES

Immuno-therapeutic potential of *Schistosoma mansoni* and *Trichinella spiralis* antigens in a murine model of colon cancer

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Summary

Considerable evidence indicates a negative correlation between the prevalence of some parasitic infections and cancer and their interference with tumor growth. Therefore, parasitic antigens seem to be promising candidates for cancer immunotherapy. In this study, the therapeutic efficacy of autoclaved *Schistosoma mansoni* and *Trichinella spiralis* antigens against a colon cancer murine model was investigated. Both antigens showed immunomodulatory potential, as evidenced by a significant decrease in serum IL-17, a significant increase in serum IL-10, and the percentage of splenic $CD4^+$T-cells and intestinal $FoxP3^+$ Treg cells. However, treatment with *S. mansoni* antigen yielded protection against the deleterious effect of DMH-induced colon carcinogenesis only, with a significant decrease in the average lesion size and number of neoplasias per mouse. For the first time, we report an inhibitory effect of *S. mansoni* antigen on the progression of chemically induced colon carcinogenesis, but the exact mechanism has yet to be clarified. This anti-tumor strategy could introduce a new era of medicine in which a generation of anticancer vaccines of parasitic origin would boost the therapy for incurable cancers.

Keywords Autoclaved antigen vaccine · *Schistosoma mansoni* · *Trichinella spiralis* · Colon cancer · Immunotherapy · $FoxP_3^+$ Treg cells

Abbreviations

ASMA	Autoclaved *Schistosoma mansoni* antigen
ATSA	Autoclaved *Trichinella spiralis* antigen
DMH	1,2-dimethylhydrazine
$FoxP_3^+$ Treg cells	Forkhead box positive T regulatory cells
CIS	Carcinoma in situ
TGF-β	Transforming growth factor-beta

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Introduction

Biological therapy marks a new era in cancer treatment designed to boost the body's natural defenses to fight cancer. The landmark discovery of tumor antigens shed light on this new therapeutic potential through stimulating the tumor suppressor immune response. Several autologous and allogeneic tumor cell vaccines have shown early promise in treating cancer patients. However, obstacles to clinical success have arisen, including immune tolerance, weak antigenic nature, and active immune evasion mechanisms employed by progressing tumors [1, 2]. To overcome these challenges, novel treatments that either boost the body's immune system in a general way or help to train the immune system to specifically attack cancer cells are urgently needed.

The immune system may not always recognize cancer cells as foreign, and even if recognized, the response may not be strong enough since these cells may give off substances that keep the immune system in check. Alternatively, the immune system is much better at recognizing and attacking germs than cancer cells, as germs are often easily seen as foreign [3]. Considerable evidence indicates that certain parasitic

infections induce anti-tumor activity either in human or in experimental animals. This was justified by a documented negative correlation between the prevalence of some parasitic infections and cancer. Furthermore, the survival rate of some cancer patients infected with certain parasites was longer than non-infected patients. [4, 5].

However, the use of parasitic infection to induce anticancer activity was limited by the virulence of live parasites and their induced morbidity. Alternatively, several attempts to use live vaccination strategies including non-pathogenic parasites have attracted significant attention [3, 6–8]. The mechanisms of tumor resistance induced by parasites are still controversial, but different mechanisms, such as raising innate or acquired immunity, the induction of anti-angiogenesis, enhancing apoptosis, or presenting common antigens, may be engaged. It has been shown that cancer-associated mucin-type O-glycan compositions are shared by certain parasites. This may also explain why only some parasites that are rich in glycosylated antigens are more likely to show anticancer activities in combating cancer immune tolerance [3, 7].

In this context, the present authors previously reported that autoclaved parasitic antigens induced a high level of homologous protective immunity. This antigen contains all antigenic components of the parasite, resulting in better stimulation of the immune system, resembling what occurs in the natural course of infection. Analysis of the autoclaved *Schistosoma mansoni* antigen (ASMA) revealed its high carbohydrate and protein contents, which could explain its high immunogenicity [9, 10]. Moreover, it was reported to be safe, stable and offered considerable longevity in a mouse model [11]. Of particular concern, *S. mansoni* expresses Tn and TF antigens, which are among the most specific human cancer-associated antigens [12, 13]. Similarly, autoclaved *Trichinella spiralis* antigen (ATSA) showed promising results and induced a high level of homologous protective immunity in a murine model, raising interest in its strong immunogenic potential [14, 15].

In view of the new era of using parasitic antigens for cancer immunotherapy, it seems interesting to investigate the therapeutic efficacy of autoclaved parasitic antigens against cancer. As we are in the early stages of understanding this relationship, we designed this study using autoclaved parasites prepared from infective stages of either *S. mansoni* or *T. spiralis* in an experimental model of colon cancer.

Material and methods

Animals

Experiment was carried out on 90 female CD1 Swiss albino mice 4–5 weeks old (20-25 g), purchased from 6th October University's animal house, Egypt. They were housed (10/cage) under standard laboratory conditions in the animal house of Medical Parasitology Department, Alexandria University. All procedures and animal treatments were in compliance with the ARRIVE guidelines for care and use of laboratory animals and were approved by the Ethics Committee of the Faculty of Medicine, Alexandria University (Protocol approval n°: 0302380).

Parasitic antigens

For preparation of ASMA and ATSA, the life cycle of both *S. mansoni* [16, 17] and *T. spiralis* [18, 19] were maintained at the Medical Parasitology laboratory. The autoclaved antigens were prepared from the infective stages of both parasites: the cercariae for *S. mansoni* and the larvae for *T. spiralis,* as previously described by Eissa et al., 2016 [20]. The protein content of both antigens was estimated according to the method of Lowry et al., 1951 [21].

Cancer induction

For induction of colon neoplasm, mice were injected subcutaneously with freshly prepared 1,2-dimethylhydrazine (DMH, Sigma Aldrich-USA) in 0.001 M EDTA, pH 6.5 in a weekly dose of 20 mg/kg for 12-week [22], whereas, EDTA was injected, as vehicle, in normal control mice. All injections were performed between 9 and 10 *a.m*. Close daily observation of mice was performed for pre-set human endpoints, including signs of morbidity; weight reduction, elevated body temperature, disturbed bowel habit, any external tumor appearance, or impaired ambulation. Survival rate was followed throughout the study.

Experimental design

Mice were randomly allocated into two groups (20/group). They received parasitic antigens intradermally over the sternum in double doses of 5 μg/kg for ASMA [23] or 70 mg/kg for ATSA [24] two weeks apart, starting after the 12th week of cancer induction. Three random control groups (10/group) served as parasitic antigens and normal controls. A group of 20 mice served as a cancer control group. All animals were euthanized at the 20th week from the first dose of cancer induction, whereas those of normal and antigen control groups were euthanized at the 8th-week from the first dose of vehicle or antigen administration. After anesthesia with thiopental sodium (45 mg/kg, *ip*), blood was collected for biochemical analysis, and then animals were euthanized by an overdose of thiopental. Mice that reached one of the human endpoints were euthanized immediately and replaced. The experiment was replicated to confirm the findings. Data presented herein are the averages of both experimental replicates.

Biochemical analyses

The immune profile was assessed by estimation of serum IL17, IL-10, TGF-β, percentage of splenic $CD4^+$ T-lymphocytes, and number of forkhead box $(FoxP3)^+$ Treg cells in colonic tissue.

Serum cytokines

Blood samples were collected by cardiac puncture, centrifuged at 1000 g for 10 min, and the sera were collected and stored at −80 °C. Serum concentrations of IL-17, IL-10, and TGF-β were determined using mouse ELISA kits (eBioscience, San Diego-USA), according to the manufacturer's instructions.

Splenic $CD4^+$ T-cells

T-lymphocytes were expressed from splenic suspensions, washed, and separated by differential centrifugation using sterile Ficoll-Hypaque. Viable lymphocyte mononuclear cell layer collected was incubated with anti-mouse $CD4^+$ and $CD3^+$ monoclonal antibodies labelled with FITC (fluorescein isothiocyanate) and PE (Phycoerythrin), respectively (BioLegend Inc., USA). Percentage values for $CD3^+CD4^+$ T lymphocytes were determined [25].

Histopathological assessment

Complete necropsies were performed for all animals and were blindly assessed. All organs were examined macroscopically and were fixed in 10% buffered formalin. For each animal, the whole gastro-intestinal tract (GIT), including lesional and non-lesional areas, was sectioned. Apart from GIT, representative sections from each organ were sampled for histological examination with haematoxylin and eosin (H&E). A semi-quantitative grading was done to assess intestinal dysplasia [26], intestinal lymphocytic infiltration, as well as, liver cell dysplasia [27]. The size of the colonic tumor was determined microscopically by measuring its diameters using an ocular micrometre. The volume was calculated using the following formula: $V = (ab^2)/2$, where 'a' is the long axis, and 'b' is the short axis, and expressed in μm^2 [5].

Immunohistochemistry for regulatory T cell (Tregs)

The number of $FoxP3^+$ Treg cells was analysed in colonic tissue by immunohistochemistry. Paraffin-embedded serial colonic tissue sections (4-μm) were stained with a 1:100 dilution of affinity purified anti-mice Foxp3 monoclonal antibody (eBioscience, San Diego, USA) followed by incubation with the biotinylated secondary antibodies (Dako, 1:200). Staining was developed using an avidin-biotin-based detection system and visualized with peroxidase or DAB (Dako REAL™ EnVison™ Detection System-Denmark A/S). Tonsils were used as a positive control for each run and negative controls were performed by omitting primary antibodies. Ten, randomly selected high-power fields (HPFs ×400) were analysed for $FoxP3^+$ lymphocytic infiltrate, and positive cells were expressed as the mean number of positive cells/10 HPF. This assessment was done according to the method of Kim et al., 2013 [28] after modification.

Statistical analyses

Parametric data were analysed by one-way analysis of variance (ANOVA) followed by Least Significance Difference (LSD) criterion for multiple comparisons and presented as means ± S.E.M. Non-parametric data were compared by Kruskal-Wallis test followed by Mann-Whitney U-test for mean ranks between groups. Survival was evaluated from day of cancer induction until euthanasia as percentage. Data were processed using Graph-Pad Prism software (version 7.0). Data presented are the average of two experimental replicates. Statistical Significance was set at $P < 0.05$.

Results

Survival rate

Compared with normal control mice, DMH-injected mice were generally in good condition and gained weight. Eighty percent of the ASMA-treated cancer mice survived the 20th week of the study. The least survival percent was in the ATSA-treated cancer group (60%) (Table 1).

Serum cytokines

The serum concentration of IL-17 was significantly increased in cancer control versus normal mice. In DMH-injected mice, both antigens significantly decreased serum IL-17 versus cancer control mice. Contrarily, the serum concentration of IL-10 was significantly increased in both ASMA- and ATSA-treated cancer mice versus cancer controls, while that of TGF-β was not significantly increased. The administration of both antigens (ASMA and ATSA) without cancer induction significantly increased IL-10 and TGF-β serum concentrations versus normal controls. The increase in serum IL-10 in ASMA controls was highly significant versus both normal and cancer control mice, while the increase in serum TGF-β in ATSA controls

Table 1 Effects of antigens treatment on neoplasia in mice injected with DMH for 12-week and sacrificed at the 20th week

Groups	Cancer control	ASMA-treated cancer	ATSA-treated cancer	ASMA control	ATSA control	Normal control
Total/effective number[a]	20/14 (70%)	20/16 (80%)	20/12 (60%)	10/8 (80%)	10/8 (80%)	10/9 (90%)
TBA%[b]	71.43 (10)	56.25 (9)	91.67 (11)	–	–	–
Lymphocytic infiltration[c]	1.4	3.3[#]	2.4[#$]	–	–	–
Tubular adenoma[d]	+++	–	–	–	–	–
Anal SCC (μm^2)	177.14	58.71	–	–	–	–

TBA, tumor-bearing animals; *SCC*, squamous cell carcinoma; *LCD*, liver cell dysplasia. [#] $p < 0.05$ versus cancer control, [$] $p < 0.05$ versus ASMA-treated cancer mice.

[a] Total number of mice at the beginning of study/Number of mice surviving 20-week after beginning of DMH administration (survival %)

[b] Percentage

[c] Mean rank

[d] Grading (+)

was highly significant versus cancer controls and cancer-treated mice (Fig. 1a–c, respectively).

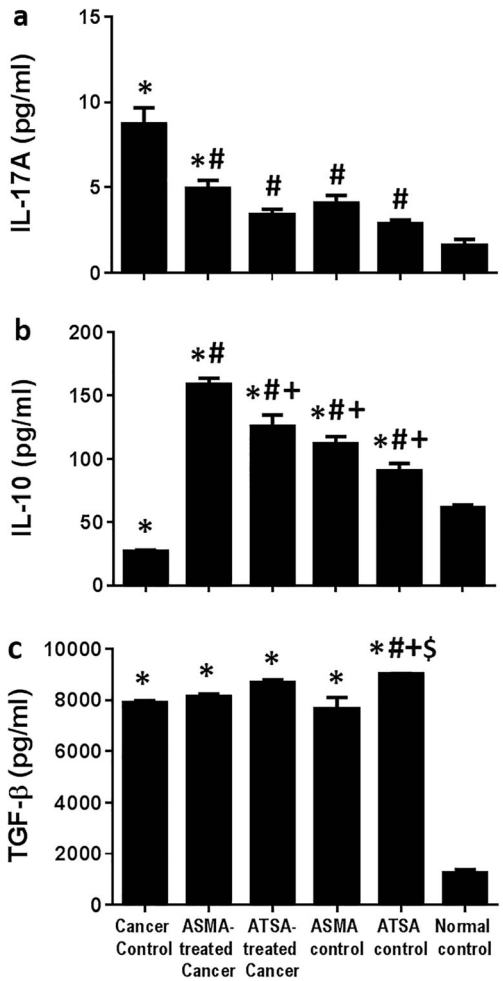

Fig. 1 Effect of ASMA or ATSA treatment in DMH-induced colon cancer on serum cytokines; (**a**) IL-17, (**b**) IL-10, and (**c**) TGF-β. Data are expressed as the means ± S.E.M. [*] $p < 0.05$ versus normal control, [#] $p < 0.05$ versus cancer control, [+] $p < 0.05$ versus ASMA-treated cancer mice, and [$] $p < 0.05$ versus ATSA-treated cancer mice. n: number of mice/group

Splenic CD4$^+$ T cell count

The percent of splenic CD4$^+$ T cells in cancer control mice was significantly lower versus normal controls. However, mice treated with both antigens showed an increased percentage of splenic CD4$^+$ T cells versus normal controls, significantly higher in mice receiving ASMA, whether cancer bearing or controls (Fig. 2a).

Number of regulatory T cells (Tregs) expressing the FoxP3

Positive FoxP3 cases showed nuclear staining in colonic lymphocytes (Fig. 3a-f). Colonic sections from cancer control mice showed a minimum range of activated Treg cells that was non-significantly increased versus the normal colon. The DMH-injected mice treated with either ASMA or ATSA showed a significant increase in FoxP3$^+$ Treg cells versus cancer controls. Additionally, there was a significant difference between the two antigen-treated groups in favor of ASMA treatment.

Gross pathological findings

In tumor-bearing animals, varying volumes of ascetic fluid were noticed. The wall of the large intestines, especially the descending colon, showed variable constrictions and scattered small polypoid projections in the lumen. No gross abnormalities were detected in other organs. A mass was seen fungating from the anal canal of one animal in the cancer controls, and a smaller non-fungating mass was seen in another animal in the ASMA-treated cancer group.

Histopathological findings

The effect of treatment with ASMA and ATSA on the incidence of neoplasia (number of tumor-bearing animals), types

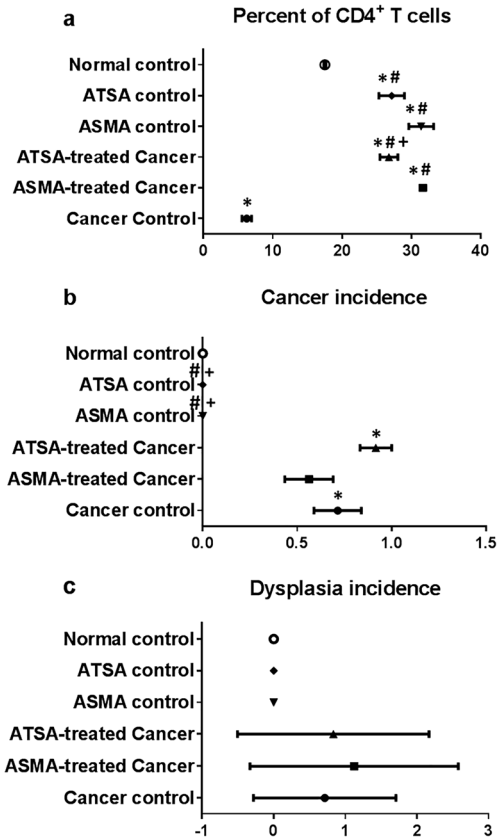

Fig. 2 Effect of ASMA or ATSA treatment in DMH-induced colon cancer; **(a)** percent of splenic $CD4^+$ T cells, incidence of **(b)** cancer, and **(c)** dysplasia in mice. Data are expressed as the means ± S.E.M in (a), and as the mean ranks in (b, c). $^{*}p < 0.05$ versus normal control, $^{\#}p < 0.05$ versus cancer control, and $^{+}p < 0.05$ versus ASMA-treated cancer mice. The number of mice was 14, 16, 12, 8, 8, and 9 in vehicle-treated, ASMA- or ATSA-treated cancer groups, ASMA or ATSA controls, and normal controls, respectively

of colonic neoplastic changes [tubular adenoma, dysplasia, carcinoma in situ (CIS)], average lesion size, number of neoplasms per colon (multiplicity), lymphocytic infiltration, and extra-intestinal lesions [liver cell dysplasia (LCD), anal squamous cell carcinoma] in DMH-injected mice are shown in Table 1 and Fig. 4a-f. Regarding colon cancer, the most observable inhibitory effect on DMH-induced neoplastic changes was found in ASMA-treated mice. Although the decrease in the incidence of neoplasia and dysplasia in ASMA-treated mice was non-significant versus cancer controls (Fig. 2b, c, respectively), there was a significant decrease in the average lesion size and number of neoplasias per mouse (Fig. 5a, b, respectively), which were highly significantly lower versus mice-treated with ATSA. The latter group not only provided a non-significant difference in neoplasia incidence and multiplicity versus cancer controls, but their average lesion size was significantly higher than cancer controls. Regarding extra-intestinal neoplasia, mice treated with ASMA exhibited the lowest incidence of LCD (Fig. 5c).

Discussion

Colorectal cancer (CRC) is a potentially immunogenic tumor and one of the leading causes of cancer-related morbidity and mortality worldwide. An effective therapy still does not exist, which creates the need for a promising cancer immunotherapy [29]. Being an inflammatory-driven malignancy, the CRC microenvironment plays a pivotal role in its behavior through the involvement of innate immunity. Conversely, evidence supports the role of adaptive immunity in cancer immunosurveillance [30].

Notably, our data demonstrated that the autoclaved *S. mansoni* crude antigen (ASMA) but not that of *T. spiralis* (ATSA) can provide tumor protective activity against a murine model of colon cancer. The administration of ASMA either prophylactically (unpublished data) or therapeutically showed significant inhibitory effects on DMH-induced neoplastic changes.

Regarding CRC, ASMA treatment led to a significant decrease in the average lesion size and number of neoplasias per mouse versus cancer control and ATSA treatment, yet its cancer incidence non-significantly decreased. Moreover, ASMA-treated mice exhibited the highest survival rate and the lowest incidence of extra-intestinal neoplasia, while both antigens revealed a significant increase in intestinal infiltrating lymphocytes versus cancer controls. Immunologically, ASMA treatment induced an innate immune response, triggered the immune regulatory arm via increasing colonic expression of $FoxP3^+$ Tregs, and significantly decreased the serum IL-17 level. Unexpectedly, while ATSA administration led to an immunological response comparable to that of ASMA, but of a significantly lower level, this response was not reflected histo-pathologically in tumor protective activity.

While conflicting evidence in humans and experimental animals links schistosomiasis with colon carcinogenesis, the association of *S. mansoni*, in particular, to CRC occurrence is non-justified [31]. From different perspectives, to the best of our knowledge, we report for the first time a protective effect of the *S. mansoni* antigen against chemically induced colon carcinogenesis. Unravelling the mechanistic mystery by which parasites boost the immune system against tumors is still the main challenge.

Several hypotheses could explain ASMA's observed interference with tumor growth. The present findings denote that ASMA treatment led primarily to a non-specific immune stimulation with a Th2-dominant response, as demonstrated by a significantly higher frequency of $CD4^+$ T cells and IL-10 and a non-significant increase in TGF-β. Generally, helminthic infection is known to evoke a host's immune response to the Th2 phenotype, marked by the production of cell-derived mediators (IL-4, 5, 10 and 13, and TGF-β to overcome the inflammatory activity of the host's Th1 response to secure parasite survival [32]. It is assumed that this response is

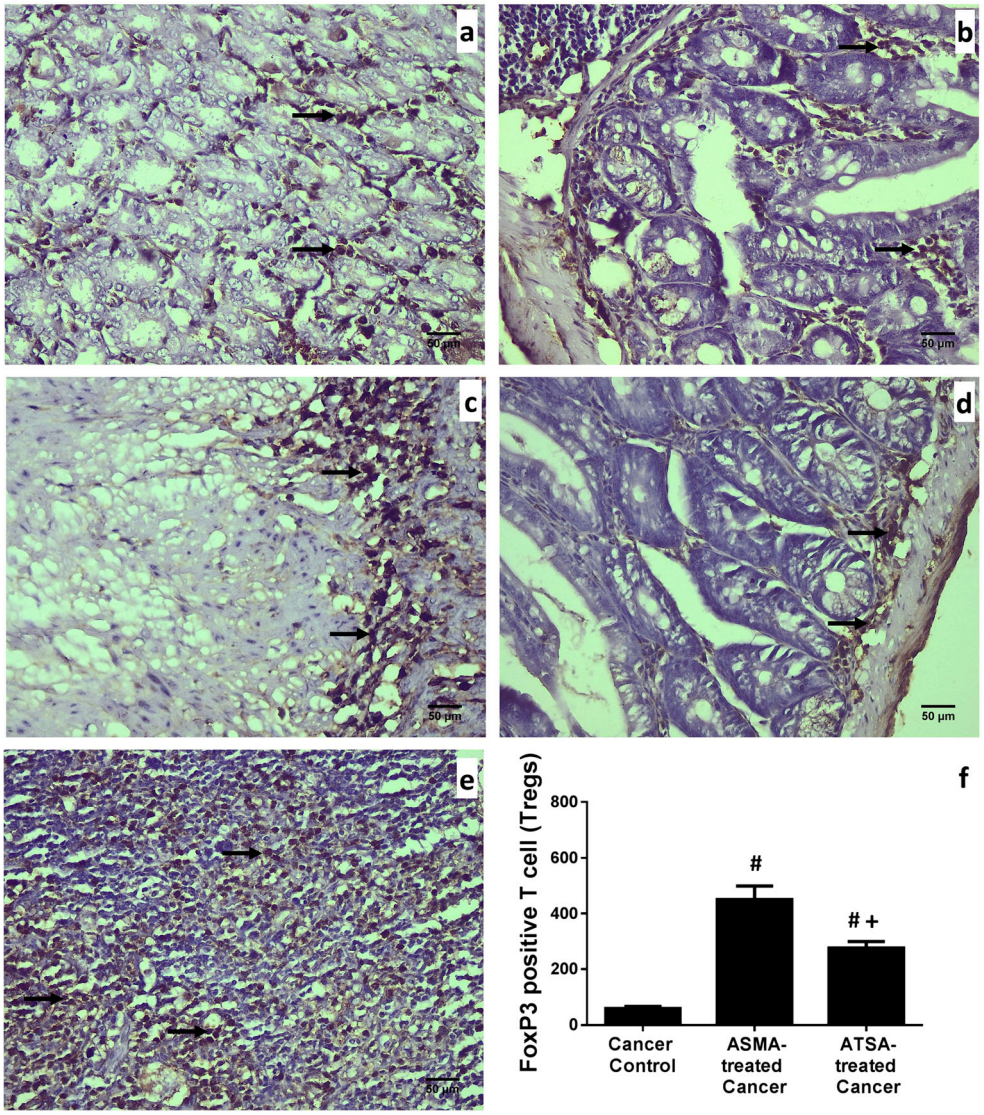

Fig. 3 Representative photomicrographs of mice colonic sections stained immunohistochemically with the anti-foxp3 antibody showing scattered FoxP3$^+$ Treg cells (arrows) in (**a**) ASMA-treated cancer, where positive nuclear staining of almost all mucosal lymphocytes is seen, (**b**) ATSA-treated cancer, where positive nuclear staining is noted in most mucosal lymphocytes and in some of the lymphocytes in the lymphoid aggregate [upper left], (**c**) ASMA control and (**d**) ATSA control mice, where in both a moderate increase in FoxP3$^+$ T cells number is shown, and (**e**) vehicle-treated cancer control, where positive nuclear staining is seen in almost 50% of the mucosal lymphocytes. In (**f**) number of FoxP3$^+$ Treg cells in immunohistochemically stained intestinal sections. Data are expressed as means ± S.E.M. $^{\#}p < 0.05$ versus cancer control, and $^{+}p < 0.05$ versus ASMA-treated cancer mice. Scale bar = 50 μm

intermingled with an increased expression of the FoxP3$^+$ Treg cells that are known to regulate Th1-driven inflammation through IL-10- and TGF-β-dependent responses. These suppressive cells have been implicated as potential regulators of helminth-induced immune responses.

Therefore, it seems that upon helminthic crude antigen administration, a constant antigen-dependent stimulation of naïve or memory CD4$^+$ T cells in the presence of primarily IL-10 (Th2-derived) leads to the generation of IL-10-producing FoxP3$^+$ suppressive Treg cells. The anti-inflammatory IL-10 as well as the immunomodulatory TGF-β both play a major role in the full suppressive potency of Tregs and the creation of a tolerogenic cytokine environment. Notably, Tregs not only restrict the access and activation of T effector (Teff) cells by various mechanisms but are also capable of killing these immuno-reactive cells in a uniform fashion, encompassing the whole inflammatory process [33, 34]. Thus, controlling cancer-driven inflammation is expected to restore epithelial homeostasis and intestinal integrity, thereby combating tumorigenesis. Earlier studies reported an expansion of both Treg and Teff cells, limiting both Th1 and Th2 responses, in an IL-10-dependant manner in mice immunized with *S. mansoni* eggs [35, 36].

The role of the suppressive Tregs in CRC growth and progression is controversial. Although the concept of an 'inflammation-dysplasia-carcinoma sequence' highlights the role of chronic inflammation in CRC incidence, physiological countermeasures implement a local Treg infiltration that controls inflammation. This protection is thought to be boosted herein by a concomitant helminth-induced immune response. Contrarily, under some circumstances, this Treg-induced self-tolerance is skewed towards suppressing tumor-specific immune responses and establishing an immunosuppressive tumor microenvironment, enabling tumor immune evasion. This effect is evident in many inflammatory conditions where Tregs can convert into pro-inflammatory IL-17-producing

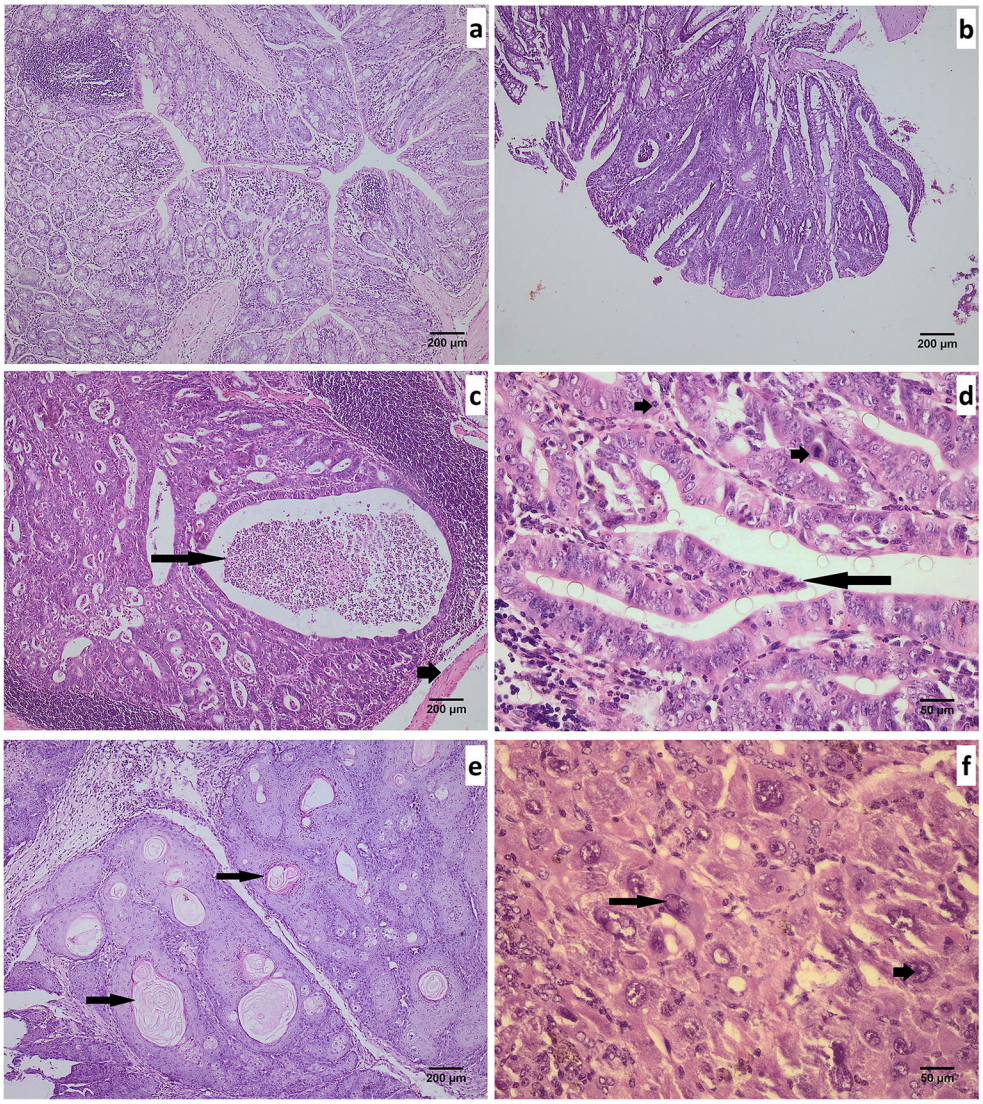

Fig. 4 Representative H&E-stained sections in different groups of DMH-induced cancer colon in mice (a through d) show progressive colonic lesions from hyperplastic mucosal lining showing polypoid projections inside the colonic lumen **(a)**, to adenomatous polyp showing increased number of glands and cells per unit area, with enlarged hyperchromatic nuclei and marked decrease in mucin production **(b)**, to carcinoma-in-situ showing closely packed glands lined by cells with hyperchromatic pseudostratified nuclei. Most glands contain necrotic debris in their lumina [arrow]. Note that the carcinoma doesn't extend to the musculosa [arrow head] **(c)**. A high power of carcinoma-in-situ **(d)** shows focal villous configuration [arrow] and abnormal mitotic figures [arrow heads]. Representative H&E-stained sections for extra-intestinal lesions (e & f), where **(e)** shows an anal well-differentiated squamous cell carcinoma composed of sheets and nests of squamoid cells with abundant pink cytoplasm, mild nuclear atypia and well developed keratinization [arrows]. Liver section **(f)** shows markedly dysplastic hepatocytes in the form of large irregular hyperchromatic nuclei, prominent nucleoli [arrow head], coarse chromatin and pseudonuclear inclusions [arrow]. Scale bar = 200 μm in (**a**, **b**, **c**, & **e**) Scale bar = 50 μm in (**d** & **f**)

(Th-17) cells that have been linked to cancer initiation. Otherwise, it has been speculated that Tregs may initially hinder tumorigenesis by controlling inflammation, but once the tumor is well established and no longer dependent on pro-inflammatory stimuli, they may turn against the host by hampering an effective specific anti-tumor response [37].

Overall, despite some prognostic controversies due to the various stages of CRC, the intra-tumoral and systemic accumulation of Tregs suggest its strong involvement in antitumor control. This is in line with the majority of CRC murine models and clinical data from CRC patients who related the intra-tumoral $FoxP3^+$ density to either better overall survival or treatment response [38, 39]. This evidence could support the observed ASMA protective activity against CRC progression that was further documented with a significant reduction in the CRC promotor cytokine IL-17.

Evidence reveals that IL-17 can promote tumor initiation and progression in most cancers. Various mechanisms are proposed for IL-17 pro-tumor activity, including the facilitation of the proliferation and survival of malignant cells by

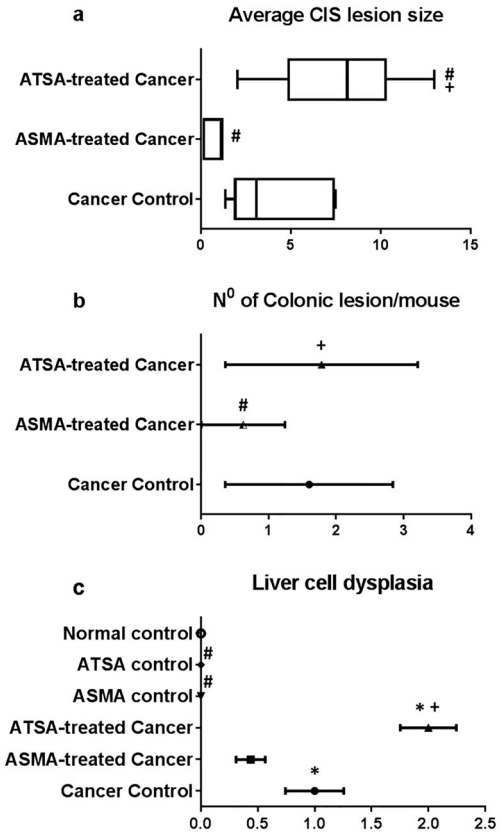

Fig. 5 Effect of ASMA or ATSA treatment in DMH-induced cancer colon on; **(a)** average CIS lesion size (μm^2), **(b)** number of colonic lesion/mouse, and **(c)** incidence of liver cell dysplasia. Data are expressed as means ± S.E.M in (**a**), and as mean ranks in (**b**, **c**). $^{*}p < 0.05$ versus normal control, $^{\#}p < 0.05$ versus cancer control, and $^{+}p < 0.05$ versus ASMA-treated cancer mice. Number of mice was 14, 16, 12, 8, 8, and 9 in vehicle-treated, ASMA- or ATSA-treated cancer groups, ASMA or ATSA controls, and normal control, respectively

boosting inflammation-associated signaling pathways and stimulating glycolysis and growth factors in CRC cells, the suppression of cytotoxic cells-mediated immune-surveillance against tumors, and the promotion of tumor metastasis by fostering angiogenesis. Accordingly, an elevated IL-17 expression level in the serum and tissue of CRC patients is a promising sensitive prognostic indicator for CRC [40].

The lack of an ATSA tumor protective response remains to be elucidated, despite its documented immunomodulatory role in various allergic and autoimmune diseases [15, 20]. Mice treated with ATSA showed a non-significant increase in neoplasia incidence and multiplicity versus cancer controls, and their average lesion size was significantly higher than cancer controls and ASMA-treated mice. Immunologically, ATSA treatment led to a less significant increase in $CD4^+$ cells, IL-10, and $FoxP3^+$ Tregs with a less significant decrease in IL-17 levels and a non-significant increase in TGF-β versus ASMA-treated mice.

Contrarily, experimental data support a promising role of *T. spiralis* invasion in mediating effective resistance to tumor cell expansion in various cancer cell lines [6, 24]. This difference in targeting cytokine regulation, observed herein between ASMA and ATSA, may be related to their different habitats and surface antigens that could explain their different tumor response. While *S. mansoni* evolved to resist host immunity and its derived antigen induces immune tolerance by a Th2-dominant response to ensure its host persistence and control of inflammation, *T. spiralis* host evasion is thought to initially confer host immunomodulation, inducing mucosal immune responses in favor of parasite expulsion through a complex transition in the Th1/Th2 cytokine profile, inducing immediate-hypersensitivity reactions [41]. The coincidence of ATSA's initial immune-stimulant response with the carcinogen-induced inflammatory effect could be responsible for the observed promotion of CRC growth.

Paradoxically, the significant increase in TGF-β by ATSA per se could be a reasonable mechanism behind its CRC-promoting activity. In normal intestinal epithelium, TGF-β serves as a tumor suppressor by inhibiting cell proliferation and inducing apoptosis. However, its signaling pathway is reported to play a central role in the predisposition and progression of CRC [42]. The switch of the TGF-β growth suppressor to oncogenic activity is not well understood. Is it related to cytokine concentration, the dysregulated expression of components in its signaling pathway, the extent of mucosal inflammation, or the stage of CRC? Interestingly, the increase in TGF-β serum concentration by *S. mansoni* was not significant, which could support the implication of TGF-β in *T. spiralis*-induced tumor promotion. Likewise, both the ASMA and ATSA control groups showed the same significant difference in cytokine profiles, even in the absence of cancer, which reflects the different immunomodulatory roles of each parasite.

From different perspectives, an interplay of a tumor-adaptive immune response could clarify these intriguing disparities in the tumor response of both parasitic antigens. The substantial emergence of shared mucin glycosylated antigens between human CRC and *S. mansoni* that are claimed to produce a cross-reacting immune response could clarify *S. mansoni*'s protective anti-tumor activity. Although this hypothesis is not explored directly herein, it is supported by the observed significant increase in intestinal infiltrating lymphocytes in mice treated with ASMA rather than in cancer controls. The density of tumor-infiltrating lymphocytes in CRC has been shown to inhibit tumor growth and is associated with improved prognosis [43].

Of the highly immunogenic mucin epitopes extracted from *S. mansoni,* the Tn antigens are expressed on both schistosomula and adult worms, and TF antigens are also expressed on the parasite surface and eggs. Remarkably, these two antigens have been previously shown to be highly sensitive and specific markers for CRC. Moreover, Berriel et al. (2005) reported the expression of the Tn antigen on DMH-induced rat colon cancer cells. Similarly, Thors et al., 2006 reported that sera from *S. mansoni*-infected mice cross-reacted with human gastric and bladder carcinoma [44–46].

This intricate relationship between parasitic and human pathologies and shared glycoprotein epitopes is still obscure. Since the Tn antigen is a non-physiological glycan structure (recognized as foreign), its abnormal expression is associated with various types of cancer and human disorders. Although the presence of the Tn antigen epitopes on the CRC surface favors tumor immune evasion and progression, it provides a powerful boost to cell-mediated immunity in case of parasitism. Generally, carbohydrate alone has limited immunogenicity; it needs to be coupled to a carrier protein to induce helper T and B-cell stimulation. Thus, it could be speculated that the exposure to the whole *S. mansoni* antigen containing the Tn protein could be a suitable immunogenic booster to cytotoxic T-cells, skewing the immune system toward an effective specific response against cancers sharing the same epitopes [47].

However, *T. spiralis* has not been reported to express glycosylated antigens, only different carbohydrate epitopes, which are not shared by human carcinoma antigens [48]. Thus, it is unlikely that the *T. spiralis* antigen could cause an adaptive immune response against cancer colon. Nevertheless, the present results reflect an important observation that each parasite seems to possess a unique tumor specificity. *S. mansoni* provides protection against CRC progression and liver cell dysplasia, while *T. spiralis* protects against anal squamous cell carcinoma. Although the incidence is scarce and non-significant, anal carcinoma appeared once in cancer controls, ASMA-treated and ASMA-prophylactic mice, and none appeared in mice exposed to ATSA. Further studies are warranted to prove the relationship between *T. spiralis* and squamous cell carcinoma and to unravel the underlying immune mechanism.

In summary, a cancer protective effect of *S. mansoni* but not *T. spiralis* crude antigens against DMH-induced murine colon cancer was demonstrated. We speculated that *S. mansoni* raises the host's non-specific immune response that might confer, at least in part, protection against tumor progression. However, since the *T. spiralis* antigen did not reveal any anti-tumor profile, even with a considerable stimulation of innate immunity, we hypothesized that *S. mansoni* glycosylated antigens can be involved in evoking a cross-reactive adaptive immune response that might be effective in hindering colon tumorigenesis. This hypothesis remains to be elucidated.

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Compliance with ethical standards

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