Cytokine 123 (2019) 154743

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

The immune response to *Hymenolepis nana* in mice decreases tumorigenesis induced by 7,12 dimethylbenz-anthracene

E. Ramos-Martínez[a,*], J. Rojas-Serrano[b], O. García-Hernández[a], F.J. García-Vázquez[c], W.A. Andrade[d], G. Avila[e], L. Salinas-Pasquier[f], M.R. López-Vancell[a]

[a] Unidad de Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[b] Servicio Clínico de enfermedades del Intersticio del Pulmón y Reumatología Instituto Nacional de Enfermedades Respiratorias, "Ismael Cosío Villegas", Ciudad de México, Mexico
[c] Instituto Nacional de Pediatría, Laboratorio de Inmunogenética Molecular, Departamento de Análisis Clínicos y Estudios Especiales, México, DF, Mexico
[d] Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil
[e] Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México. Ciudad de México, Mexico
[f] Servicio de Anatomía Patológica, Unidad de Citopatología. Hospital General de México "Dr. Eduardo Liceaga", Ciudad de México, Mexico

ARTICLE INFO

Keywords:
Hymenolepis nana
Th2 response
Tumor
DMBA
Cancer

ABSTRACT

Background: Cancer is a high-impact disease throughout the world. A negative correlation has been established between the development of cancer and the Th2 immune response. Infection by helminth parasites is characterized by the induction of a strong and long-lasting Th2 response. The aim of this work was to evaluate the effect of the immune response induced by the infection with the helminth *Hymenolepis nana,* on the tumorigenesis induced by dimethylbenz-anthracene (DMBA) in mice.
Methodology: Four different groups of 14 female BALB/c mice were formed; Group A, dimethyl sulfoxide (DMSO) (vehicle) was administered cutaneously, Group B infected with *H. nana*, group C, cutaneously DMBA and finally Group D infected with *H. nana* and cutaneous DMBA. The tumor load was determined in those animals that developed cancerous lesions. In all groups were determined: serum concentration of IgE, IFNγ, IL-10, IL-5 and malondialdehyde (MDA). The inflammatory infiltrate was analyzed from skin samples and the expression of the main eosinophilic protein and myeloperoxidase was determined.
Results: The group previously infected with *H. nana* had a reduced amount of tumors with smaller size, in comparison to the group that received only DMBA; this reduction was associated with lower levels of IFNγ and IL-10, while levels of IgE, IL-5 and MDA were higher. Further, the number of eosinophils and neutrophils was statistically higher in the animals that were previously infected with the helminth and developed less tumors.
Conclusion: The immune response induced by *H. nana* infection is associated with the reduction of tumors probably due to the activity of eosinophils and neutrophils.

1. Introduction

«Cancer» is a generic term that designates a broad group of diseases that can affect any part of the body. A defining characteristic of cancer is the fast multiplication of abnormal cells, which invade and spread to other organs, in a process termed "metastasis" [24]. This set of pathologies integrates the second cause of death in the world, causing around 8.8 million deaths in 2015 [15]. Although the factors that determine the appearance of this disease in its multiple varieties have not been fully understood, several epidemiological studies have established risk factors that are associated with the incidence of cancer, including the ones related to demographic aspects (gender, age), health history (infections characterized by chronic inflammation), feeding habits (obesity, alcohol consumption, smoking) and environmental exposures (solar radiation, mutagenic agents) [38]. One of the most common cancers nowadays is papilloma, usually associated with infection by human papillomavirus (HPV). Although most cases are self-limiting, warts sometimes develop and in some cases serious carcinomas [9]. In individuals with lesions, a strong coordinated immune response is developed by Type I (Th1) CD4+helper T lymphocytes [12], and the main immunological mediator in this type of response is IFNγ that activates cytolytic mechanisms in phagocytic cells. However, other

* Corresponding author at: Dr. Balmis No.148, Col. Doctores, Delegación Cuauhtémoc, Ciudad de México C.P. 06726, Mexico.
E-mail address: espiri77mx@yahoo.com (E. Ramos-Martínez).

https://doi.org/10.1016/j.cyto.2019.154743
Received 15 February 2019; Received in revised form 15 May 2019; Accepted 3 June 2019
Available online 27 June 2019

mechanisms favor the development of tumors, including the production of IL-10 which inhibits the activity of lymphocytes and macrophages [36].

On the other hand, some epidemiological studies have suggested that there is a strong negative association between the development of some cancers and allergic phenomenon, including protection [27]. The nature of this relationship has been explored in many studies, but the mechanisms involved have not been clarified. In several types of cancer, including pancreatic cancer, brain tumors, hematologic malignancies (such as leukemia and lymphoma), gastrointestinal cancer (stomach and colorectal) and gynecological cancer (ovarian, endometrial and cervical cancer), a negative association with allergies has been found [28]. It is well documented that the immune response to allergies is mainly orchestrated by Type 2 (Th2) CD4 + helper T lymphocytes, characterized by the production of the cytokines IL-4, IL-5, IL-9, IL-13 and IL −25 [42]. IL-4 produced by Th2 lymphocytes leads to the activation and B cells and class switch, producing large amounts of IgE, responsible for activating eosinophils, basophils and mast cells, characteristic of allergic diseases [47]. However, the Th2 inflammatory response is not exclusive of allergic diseases, since this same response is activated in the presence of extracellular parasites such as helminths. The response induced by the presence of these infectious microorganisms is similar in terms of the type of lymphocytes, the isotype of circulating antibody, the cytokines produced and the participating effectors cells, but this inflammatory response is intense and highly persistent, and excludes the development of others such as Th1 [42].

Cutaneous administration of DMBA in mice is a model widely used in the study of cancer. This chemical agent induces carcinogenesis through the induction of mutations in tumor suppressor genes such as p53 and p16, in a similar way to that induced by HPV [9,51]; even inducing a Th1 inflammatory response [46]. The transformation and immortalization induced by DMBA causes the formation of papillomas that can evolve to carcinomas and even develop metastasis [50].

Furthermore, it is well known that in mice the infection with the helminth parasite *Hymenolepis spp,* induces an intense and long-lasting Th2 immune response, despite the fact that the parasite is usually eliminated in about thirty days [55].

Due to the above, we decided to explore whether the Th2 immune response generated by the previous infection with a helminth parasite affected the tumor induced by DMBA in mice.

2. Methods

2.1. Animals and experimental design

Eight weeks old female BALB/c mice were purchased from Harlan Laboratory (Mexico City, Mexico) and were raised at the animal facility of the Unidad de Investigación en Medicina Experimental, Facultad de Medicina, UNAM; following the National Guidelines for Animal Care. Four groups of 14 mice was made and according to the treatment was assigned as follows: GROUP A) It received DMSO on the skin of the back; GROUP B) It was infected with *Hymenolepis nana*; GROUP C) Cancer was induced by the application of DMBA and GROUP D) It was infected with *H. nana* and DMBA was applied.

2.2. Infection protocol

For purification of *H. nana* eggs and infection of mice, procedure described by Berntzen and Vogewas followed [7]. Briefly, adult *H. nana* parasites were obtained from experimentally infected mice; the animals were sacrificed at 25 days of infection with an overdose of sevoflurane, the cestodes were recovered directly from the small intestine, washed 10 times with saline buffered with phosphate (PBS), macerated with a polycarbonate pistil and broken by shaking during 40 min in the presence of borosilicate beads and in a magnetic stirrer. The animals of groups B and D were infected orally with 120 oncospheres with a cannula. The infection of the animals was verified by coproparasitoscopic flotation tests with zinc sulphate at 14 days post infection, this last procedure was performed once a week for 6 weeks after infection.

2.3. Cancer induction

Carcinogenesis was induced in skin of the mice of groups C and D. The back of each mouse was shaved 2 days before the start of the experiment. Each mouse received two topical applications of 25 mg of DMBA (Sigma–Aldrich Chimie, Lyon, France) in 100 µl of DMSO at an interval of 72 h, followed by applications of 100 µl DMSO containing 4 mg of 12-O-tetradecanoylphorbol-13-acetate (TPA) (Sigma–Aldrich Chimie, Lyon, France) twice a week for 16 weeks. All the animals were sacrificed by an overdose of sodium pentobarbital at the end of the 16 weeks. Whole blood samples were collected without anticoagulant and the serum was purified by centrifugation at 2000 g for 10 min. The tumors were excised, cleaned, fixed in 10% buffered formalin, embedded in paraffin wax and processed for histopathological analysis. Tumor volume was measured using Vernier calipers using the formula [53]:

$$V = (4/3)\pi [D1/2][D2/2][D3/2].$$

where D1, D2 and D3 are the diameters of length, width and the height of the tumor [17].

The sum of the volumes of all the tumors in an animal was expressed as the Total Tumor Burden and from this parameter the comparisons between groups were made.

2.4. Quantification of total IgE

At the end of the trial a sample of blood was obtained without anticoagulant. Serum was separated by centrifugation and stored at − 20 °C. IgE levels were determined using a commercial ELISA kit (Abcam® AB157718, Mexico) according to the recommendations of the manufacturer.

2.5. Quantification of cytokines

The immune status of mice was analyzed using the quantification of circulating cytokines in the serum. This analysis determined the concentrations of IL-5, IL-10, and IFNγ (all from Peprotech™ Mexico) through ELISA. All procedures were conducted following the manufacturer's recommendations.

2.6. Measurement of serum malondialdehyde (MDA)

UPLC equipment and chromatographic conditions: The UPLC was an Acquity, Class H with Quaternary Pump and Fluorescence Detector. Separation was performed on an analytical column, 150.0 × 4.0 mm, particle size 5 µm Zorbax SB-c18 (Agilent). Data collection and processing were carried out using Empower 3.0 software. Mobile phase consisted of 25 mmol/l sodium acetate and methanol (50:50; v/v). The flow rate was set at 0.3 ml/min. The fluorescence detector wavelengths were set for excitation (λex) at 527 nm and emission (λem) at 551 nm. Standard Preparation: 1, 1, 3, 3 tetra methoxypropano stock solution (3 mmol/l) was used by diluting MDA standard in MilliQ™ water. MDA stock solution was aliquoted and stored at −20 °C.Working standards in MilliQ™ water (ranging from 6.0 − 100 µmol/l) were prepared fresh daily. Sample preparation: 25 µl of MilliQ™ water (blank), 25 µl of standard or 25 µl of serum, 175 µl MilliQ™ water and 150 µl of NaOH (2.0 mol/l) were mixed in 1.5 ml tubes. The tubes were mixed and afterwards heated at 60 °C for 20 min. The hydrolyzed sample was acidified with 200 µl 10% (v/v) o-phosphoric acid. After centrifugation at 15,000 *g* at 4 °C for 10 min, the supernatant was recovered, transferred to a 1.5 ml tube and mixed with 50 µl of 0.6% (v/v) tiobarbituric acid. The reaction was heated at 90 °C for 30 min. Finally the sample

was centrifuged at 20,000 g and 4 °C for 20 min and filtered by 0.22 μm polyvinylidene difluoride filter and 10 μl supernatant were injected onto the UPLC column.

2.7. Histopathological analysis of mouse skin

The histological analysis of the lesions of BALB/c mice from each of the groups was carried out at the end of the experiment. The skin samples were fixed in 4% formaldehyde buffered with PBS and included in a medium with purified paraffin and plastic polymers with regulated molecular weights, melting point of 56 °C enriched with DMSO. Cuttings of 2 and 3 μm thickness were made with each sample, the first cuts were stained with hematoxylin and eosin (H and E) for the description of the malignant transformations present in the tissue and the inflammatory infiltrate surrounding the tumors, this procedure was performed blindly by a specialist oncologist. The 3 μm cuts were used for immunostaining.

2.8. Markers by immunohistochemistry

Immunostaining was performed using the biotin-free protein detection system EPOS/HRP (Enhanced Polymer One Step/Horseradish Peroxidase) and for epitope unmasking, Epitope Retrieval Solution 10X concentrate Ph6 (Novocastra Leica Biossystem, New Castle Ltd, United Kindom) was used. In order to block the activity of the endogenous peroxidase, the samples were treated with 0.9% hydrogen peroxide in aqueous medium for 5 min, then the sample was incubated for 45 min with the polyclonal antibody anti Major Eosinophilic Protein (MBP), dilution 1:50 (Chemicon International, Calif. USA) and with the polyclonal antibody anti Mieloperoxiadasa, Ready to use (Biocare Medical USA). The sample was incubated with the anti-rabbit antibody of the Detection System: polymer/peroxidase Bond™ (Polymer Refine Detection) for 10 min each (Leica Biosystems Newcastle Ltd, United Kingdom), to visualize the reaction, 3,3′-deaminobenzidine-H_2O_2 was used as substrate (Biocare Medical CA USA), reaction was monitored under a microscope, the contrast was made with Gill's Hematoxylin and turned with Ammonium Hydroxide solution at 0.37 M. The marked inflammatory cells were quantified in the subepidermal dense connective tissue in each lesion and the averages of positive cells in 10 high power fields (equivalent to 400 magnifications) were compared.

2.9. Statistic analysis

To compare the incidence of neoplasia in the different groups, we used an RxC exact Fisher test. The tumoral mass and the cytokine levels of the different groups, were compared with a Kruskal-Wallis test or one way ANOVA as appropriate, according the distribution of the variable. Then after, we compare the differences between each different group with a Wilcoxon Rank-Sum test or the Bonferroni correction procedure. In this analysis, P was set as statistical significant at < 0.0083. according to the Bonferroni Correction. The expression of MPO and MPE was compared using Wilcoxon Rank Sum test, in this analysis, P was set at 0.05.All analysis are two sided and were performed using Stata V14.2 (Collage Station, Texas).

3. Results

3.1. All infected mice eliminated the parasite

The infection with *H. nana* was carried out in the animals from groups B and D, and the efficiency of this procedure was verified by the flotation technique 14 days after the inoculation of oncospheres. After this time 100% of the infected animals showed helminth eggs in their fecal samples, however 5 weeks after infection only 5 (35%) and 7 (50%) animals of group B and D respectively were positive to the test. Finally, 6 weeks after infection no *H. nana* eggs were found in any infected mice in both groups.

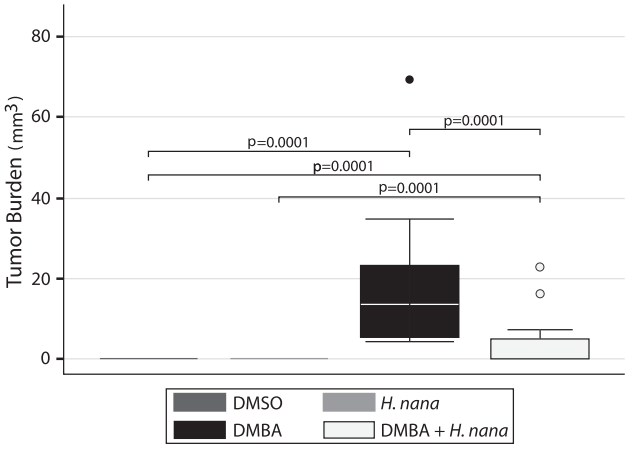

Fig. 1. Comparison of tumor burden. Comparison of the sum of the volume of the tumors developed in the animals of the groups that received DMBA in topical form in the back and those that received DMBA in topical form in the back after the infection with *H. nana*. The graphshows the mean and standard deviation; statistical differences were determined at a value of $P \leq 0.05$.

3.2. Previous infection with Hymenolepis nana reduces tumor development caused by DMBA

Six weeks after infection with *H. nana* in groups B and D, cancer was induced in mice from groups C and D, as described before. At the end of the sixteenth week after the initiation of the cancer induction protocol, animals of the four groups were sacrificed and the number and volume of tumors were determined. All fourteen animals from group C developed from 1 to 3 tumors, with volumes ranging from 0.8 to 34.7 mm^3; while in group D only 5 mice developed tumor, and it was limited to a single tumor per animal, with a range of 0.5–22.7 mm^3 (Fig. 1). Statistical analysis with Fisher's Exact Test allowed us to confirm that there is a statistically significant association between *H. nana* infection and the reduction of tumor development on the used protocol (Table 1). Furthermore, the comparison of tumor burden showed a statistically significant difference between groups C and D, where as animals that were first infected with *H. nana* and then treated with DMBA, showed reduced tumor number (Fig. 1).

3.3. Hymenolepis nana infection increases IgE levels in mice

Circulating IgE levels were determined in the serum of all animals at the end of the protocol. IgE levels did not show a statistically significant difference between the infected groups B and D (545.85 ± 20.12 ng/ml; 549.82 ± 22.70 ng/ml), but was statistically significant when compared to the non-infected groups A and C (24.62 ± 2.52 ng/ml; 23.22 ± 3.80 ng/ml) (Fig. 2).

3.4. Infection by Hymenolepis nana increases serum IL-5 levels, but not IL-10 and IFNγ

We measured the serum levels of IL-10, IL-5 and IFNγ. Serum levels of IL-10 were highly variable, but statistically significant differences

Table 1
Development of tumors in all groups of mice in the experiment.

	Group A	Group B	Group C	Group D
Animals with tumors	DMSO 0/14 (0%)	*H. nana* 0/14 (0%)	DMBA 14/14 (100%)	DMBA + *H. nana* 5/14 (35.71%)

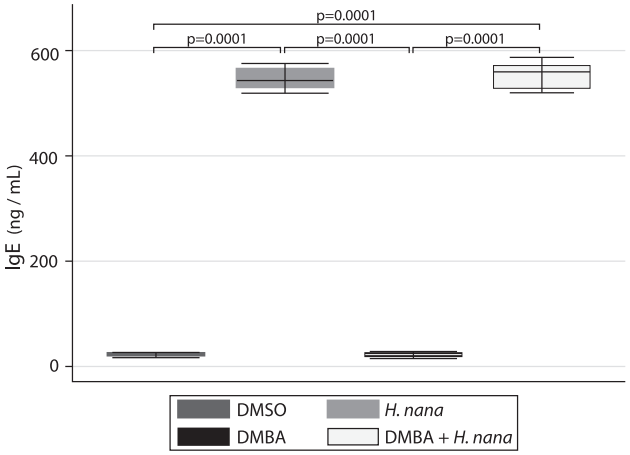

Fig. 2. Serum IgEtotal levels at the end of the experiment. Serum concentrations of IgE in all the groups. The graph show the mean and standard deviation; statistical differences were determined at a value of P ≤ 0.05.

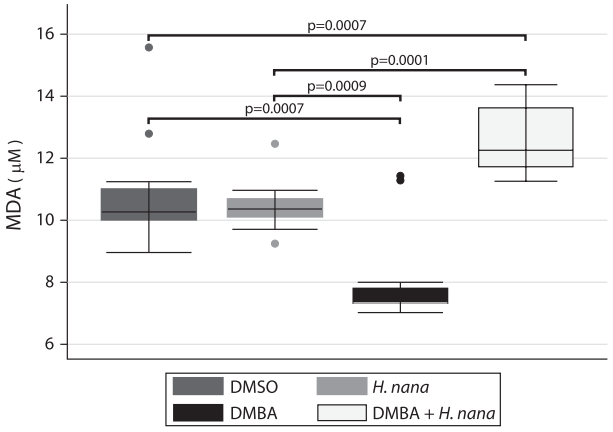

Fig. 4. Serum levels of malondialdehyde at the end of the experiments. The graph show the median and standard deviation; statistical significance was determined at $P \leq 0.05$.

were observed between all groups (Group A: 31.28 ± 5.12 pg/ml, Group B: 222.50 ± 16.63 pg/ml, Group C: 761.50 ± 28.45 pg/ml and Group D: 404.78 ± 25.19 pg/ml). The difference between groups C and D is strikingly, in both groups the cancer formation was induced by the topical application of DMBA, however the infection with *H. nana* in group D reduced the levels of this cytokine, when compared to mice only treated with DMBA (Fig. 3A). On the other hand, determination of serum levels of IL-5 showed no statistically significant differences between groups A (2.89 ± 0.46 pg/ml) and C (2.85 ± 0.47 pg/ml), nor between groups B (14.18 ± 0.6 pg/ml) and D (14.75 ± 0.86 pg/ml); nevertheless, the groups infected with *H. nana* (B and D) showed significantly higher levels of this cytokine than the non-infected groups (A and C) (Fig. 3B). Finally, the production of IFNγ in groups C (8.43 ± 0.34 pg/ml) and D (8.40 ± 0.29 pg/ml) was statistically higher than groups A (1.81 ± 0.71 pg/ml) and B (1.70 ± 0.80 pg/ml), indicating an association between the increased level of this cytokine and the application of DMBA on the back of the mice (Fig. 3C).

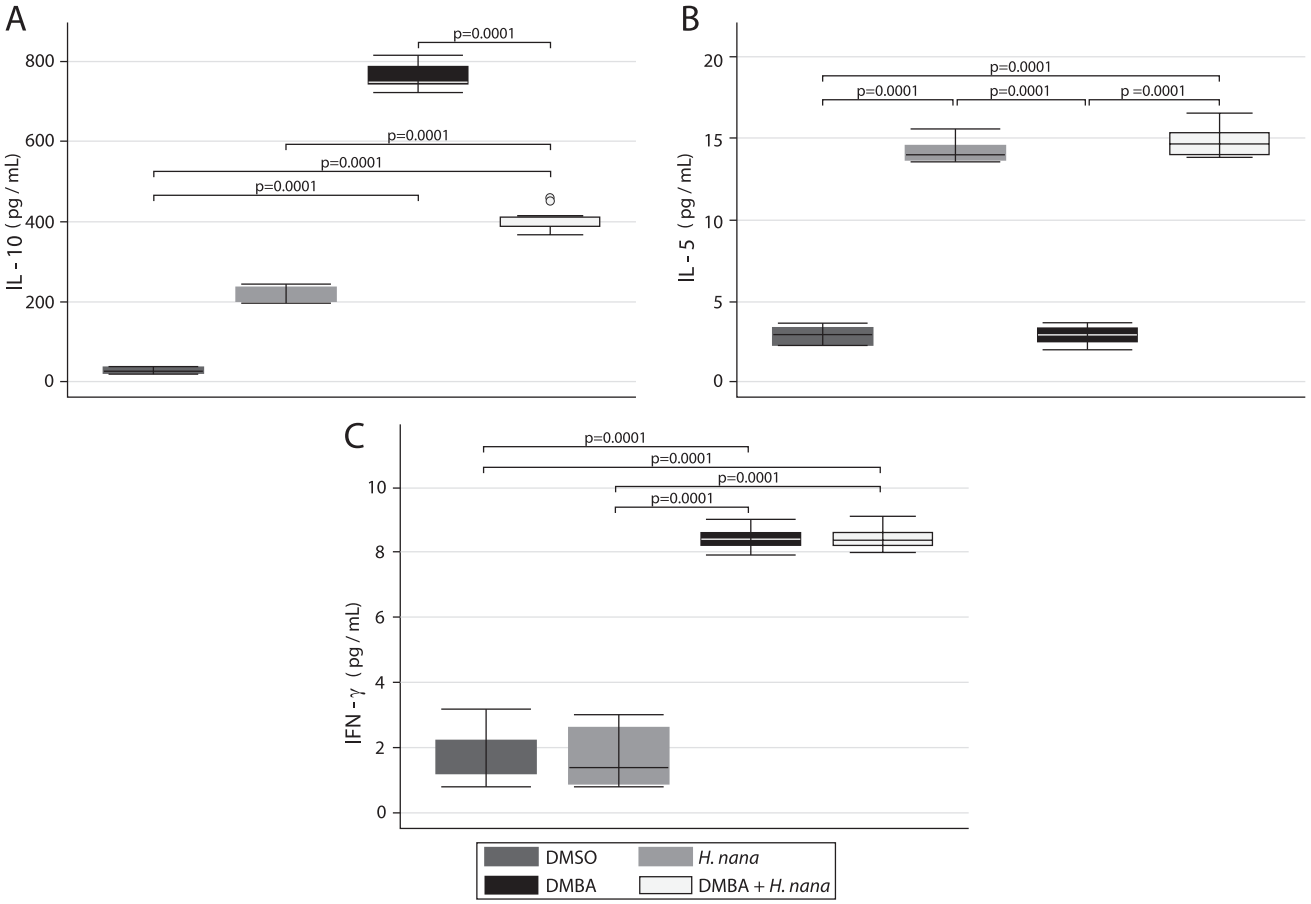

Fig. 3. Cytokines levels at the end of the experiment. Serum concentration of IL-10 (3A), IL-5 (3B) and IFNγ (3C) in all groups of mice. The graphs show the mean and standard deviation; statistical significance was determined at $P \leq 0.05$.

3.5. Previous infection with Hymenolepis nana increases malondialdehyde levels in mice treated with DMBA

Malondialdehyde is an organic compound formed as a product of oxidation. In order to evaluate the effect of oxidative stress in the animals from the different groups, this component was quantified in the serum. Malondialdehyde concentration did not show statistically significant differences between groups A (10.81 ± 1.61 μM) and B (10.35 ± 0.79 μM), but the concentrations in groups C (8.04 ± 1.42 μM) and D (12.47 ± 1.02 μM) were significantly different, being higher in the case of animals that were infected with *H. nana* and received topical DMBA (Fig. 4).

3.6. Previous infection with Hymenolepis nana reduced malignancy in mice treated with DMBA

The histopathological analysis was developed exhaustively in the skin samples of groups C and D, because only in these animals tumor development was found. Skin samples of the mice from groups A and B were excluded, because they did not development any lesion; stratified keratinized squamous epithelium without invasive lesions or presence of intraepithelial lesions was observed in the skin samples of these animals. Samples from Group C (topical DMBA), presented epithelial neoplasms with stromal invasion, rupture of the basement membrane, epidermoid and exophytic differentiation, hyperkeratosis, papillomatosis and prominent granular layer; surface layers showed coilocytic nuclear changes with enlarged hyperchromatic nuclei with irregular contours surrounded by a clear perinuclear halo. Towards the periphery of these lesions, areas of local desmoplastic reaction were observed. The inflammatory infiltrate surrounding these lesions is scarce and composed by lymphocytes and plasma cells, with the presence of eosinophils and scattered neutrophils (Fig. 5C). On the other hand, skin lesions of group D (infected with *H. nana* and topical DMBA) had minimal areas of epithelial neoplasia with epidermoid and exophytic differentiation, hyperkeratosis, papillomatosis and prominent grainy layer. The superficial layers showed coilocytic nuclear changes, with enlarged hyperchromatic nuclei of irregular contours surrounded by a clear perinuclear halo; nevertheless, the basement membrane was clearly defined, without morphological evidence of invasion in the stroma or local desmoplastic reaction. The inflammatory infiltrate surrounding these lesions was scarce and primarily composed of eosinophils and neutrophils (Fig. 5D).

3.7. Infection by Hymenolepis nana increases the number of eosinophils and neutrophils on the tumor site

Once the presence of eosinophils and neutrophils surrounding the lesions induced by DMBA was common in mice from groups C and D, we decided to evaluate the number of these cells by immunostaining using specific antibodies. In order to show eosinophils, a specific antibody was used that identified the major protein of eosinophil (Fig. 6A and B); the average of eosinophils in 10 high power fields were 22 ± 4.5 in the animals from group C, while in skin samples of animals from group D an increased number of positive cells were observed (38 ± 3 positive cells) (Fig. 6E). For the detection of cells positive for myeloperoxidase, we used and specific antibody for myeloperoxidase (Fig. 6C and D). The result showed a significant difference between skin samples from group C (170 ± 6 positive cells) when compared to samples from group D (353 ± 7.9 positive cells) (Fig. 6F).

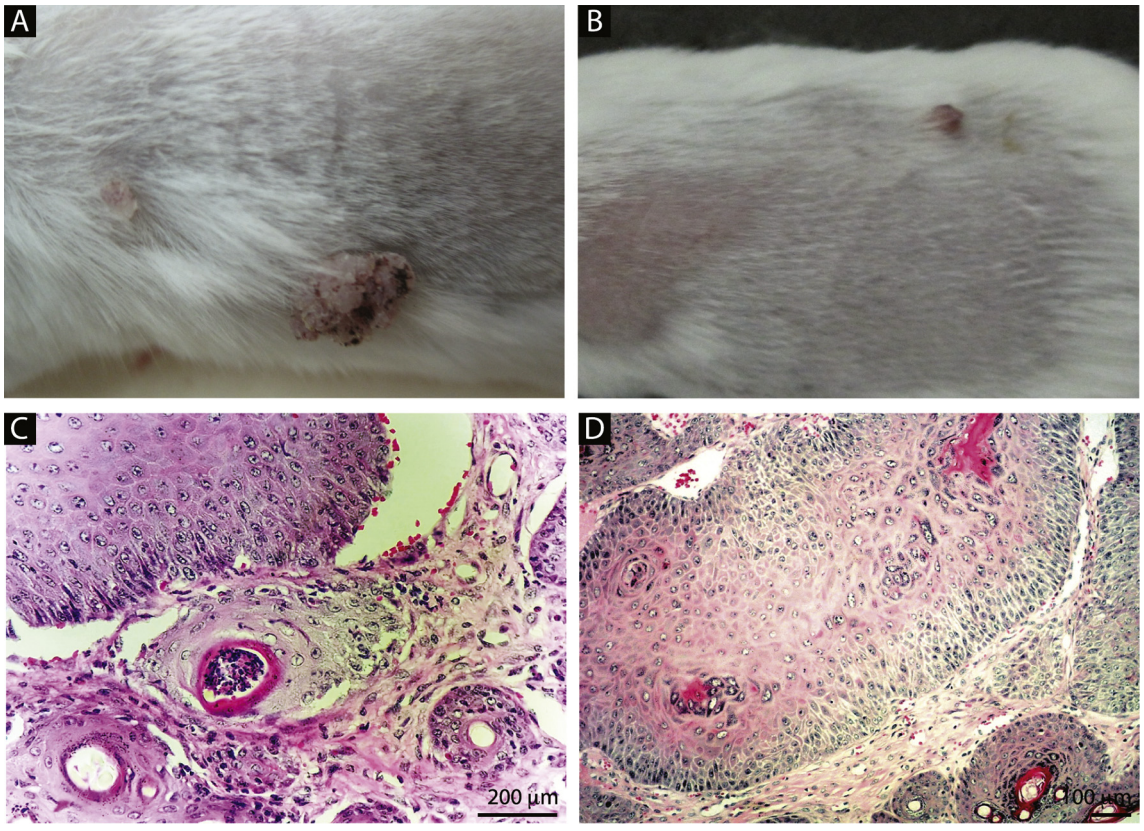

Fig. 5. Aspects of tumors induced by 7,12 dimetylbenz-anthracene. Representative image of the macroscopic aspect of the tumors developed in the group that received DMBA in the skin of the back (A) and developed by the group that received DMBA after infection with the helminth parasite *H. nana* (B). Photomicrography of the tumors developed by group that received DMBA in the skin of the back with an increase of 400X (C) and of the group that received DMBA after infection with the parasite helminth*H. nana* with an increase of 100X (D). both samples were stained by H and E.

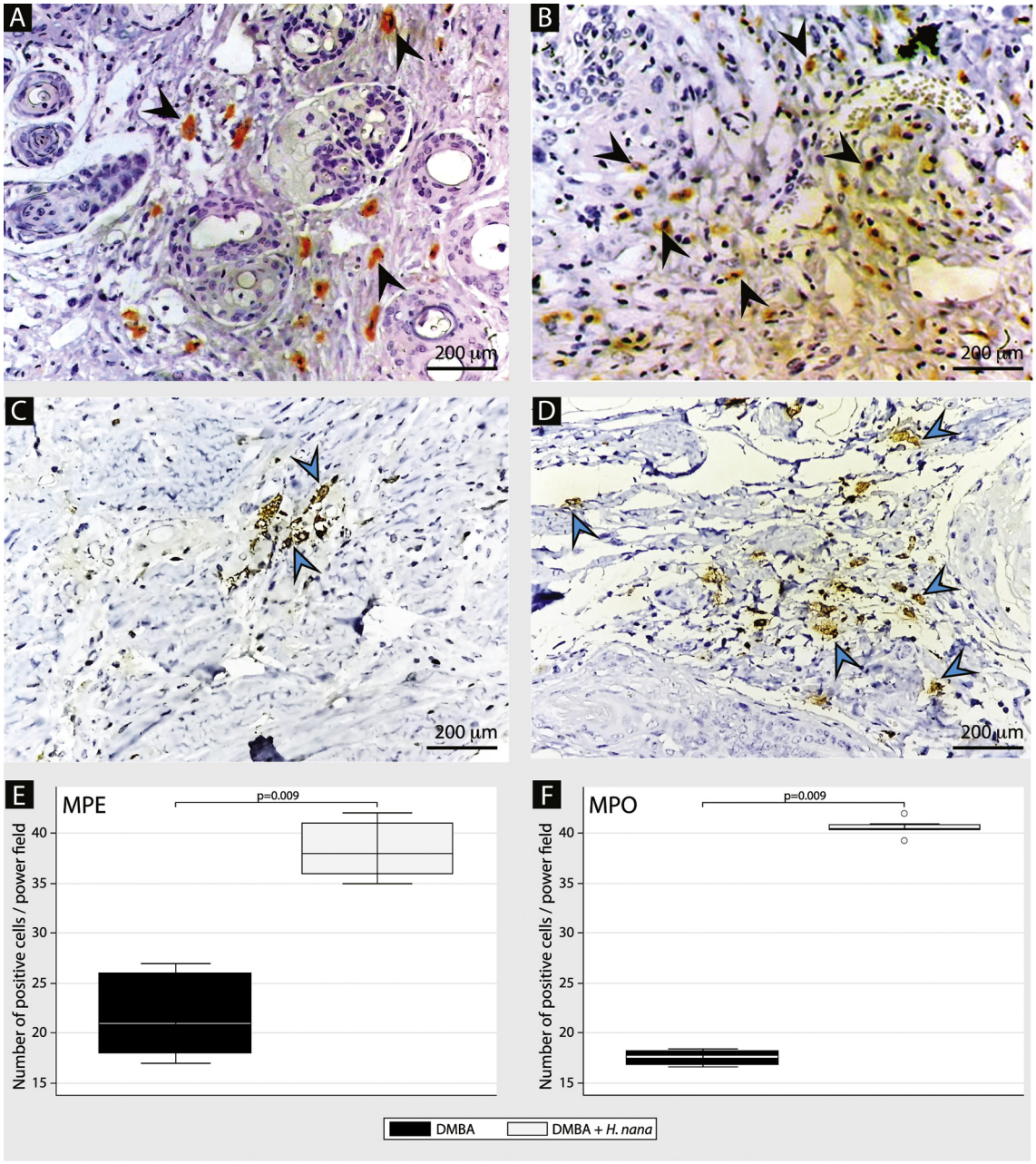

Fig. 6. Determination of eosinophils and neutrophils. Photomicrography of the major protein label of eosinophil in tumors developed by the group of animals that received DMBA on the back (A) and of the tumors developed by the animals that received DMBA after infection with the helminth parasite *H. nana* (B) black arrows signal positive cells to immunostaining. Photomicrography of the labeling of myeloperoxidase in neutrophils in tumors developed by the group of animals that received DMBA on the back (C) and of the tumors developed by the animals that received DMBA after infection with the helminth parasite *H. nana* (D) blue arrows signal positive cells to immunostaining. Comparison between the number of positive cells in the positive label of the major eosinophil protein surrounding the tumors between the two groups of mice that developed tumors (E). Comparison between the number of positive cells to the positive labeling of myeloperoxidase in neutrophils surrounding the tumors between the two groups of mice that developed tumors (F). In both comparisons graphs show the mean and standard deviation; statistical significance was determined at $P \leq 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In this study, we demonstrated that in a murine model the immune response after infection with helminth *Hymenolepis nana* reduces the development of tumors induced by the mutagenic DMBA. The possibility that this effect is due to the action of the products released by the parasite during the infection is unlikely; Avila et al., reported that circulating antigens derived from *T. solium* (cestode phylogenetically related to *H. nana*) were detectable during the first week after the animals have eliminated the intestinal parasite; since the cancer induction protocol began after that week, this reduced the possibility that some metabolite produced by the cestode acted directly on the tumor cells [5]. The elimination of helminth parasites of the *Hymenolepis* genus was explored by Weeb et al., these researchers carried out experiments in mice and determined that the inflammatory response responsible for cestode death in about 30 days was a Th2 response [55]. These results corroborate what was observed in this study, once 6 weeks post infection no animal was positive for *H. nana*. On the other hand, the immune response induced by *H. nana* infection contrasts with the one induced during the development of tumors by the application of DMBA, which

has been reported as a Th1 response with the presence of high concentrations of IFNγ [1]. This information is very interesting since the role of the Th2 inflammatory response against the development of cancer is not completely clear.

The possibility that parasitic infections or antigens derived from parasitic organisms can modulate positively the immune response and decrease the progression of diseases caused by the anomalous recognition of antigens has been previously explored in some pathologies [4,35,45,52]. Parasite cestodes in the natural host or in experimental infections are characterized by inducing a high concentration of IgE immunoglobulin [3]. This immunoglobulin is a key component in the Th2 inflammatory response, and the role played by IgE during infections by parasites has been widely described; this immunoglobulin mediates the activation of mast cells and eosinophils which promotes the elimination of these infectious organisms [43]), however, the effect of IgE on the different types of tumors continues under research. Epidemiological studies have suggested an inverse association between high concentrations of IgE in allergic patients and the development of cancer [28]. Based on observations such as mentioned before, several authors have raised the possibility of a protective effect in allergic people to certain types of cancer [28,39]. The probable mechanisms of IgE against cancer can be summarized in two different contexts: First, in the phase of sensitization with tumor-specific antigens, the activation of effectors cells characteristic of the Th2 response (mast cells, basophils and eosinophils) induces direct degranulation that would affect viability of tumor cells [32]; and second, the high concentrations of IgE directed against various antigens (even non-tumor-specific) can boost the capacity of dendritic cells to present antigens by the MHC class I processing pathway, since these antigen-presenting cells express the FcεRI [29,32]. In the present work we hypothesize that both situations can occur. Firstly, it has been documented that helminth parasites release a large amount of antigenic products during infection and some of these antigens or epitopes could induce cross-reactions with other products derived from environmental molecules or even their own [17,22]. Regarding the second possibility, by itself the presence of high concentrations of IgE ($K_a = 10^{10}M^{-1}$) could significantly increase the activity of dendritic cells, since it has been shown that the affinity of IgE for its receptor is much greater than that presented by other antibodies like IgG ($K_a = 10^{8}M^{-1}$) [23].

One of the aims of this work was to quantify cytokines as markers of the immune response to guide the search for effectors mechanisms that act against tumors. Both in animals and in humans infected with *H. nana*, the production of IL-5 is characteristic of the infection. Ajami and Rafei carried out a protocol with the purpose of determining cytokine levels in 31 patients infected with *H. nana*. The authors reported that these patients presented higher levels of IL-5 compared to individuals without helminth infection [2]. In this paper we show that the groups of mice infected with the parasite had similar IL-5 serum concentrations; however, these were significantly higher than those found in animals that were never infected. Miyaguchi et al., developed an experiment in which they use a murine model of squamous cell carcinoma in the head and neck, and reported that an experimental treatment with lactobacillus delays the development of malignancy and this phenomenon was associated with the increase of cytokines such as IFNγ, TNFα, IL-12 and IL-5 [41]. In another report, Gevariya et al. reported that treatment with omega 3 fatty acid limits the growth of prostate cancer in mice and this correlates with higher levels of IL-12, IFNγ, IL-5 and IL-13 [20]. These data corroborate our results, once the animals that were infected with *H.nana* had less tumor development and higher levels of IL-5; however, since IL-5 is an inflammatory mediator that activates eosinophils, basophils and mast cells, its relationship with the pathology would be directly related with the presence of these effectors cells in the tissue. On the other hand, IL-10 is a cytokine usually related to immunoregulation and tolerance, and plays a key role in the balance between cellular and humoral immune responses. This cytokine among many others is involved in the development and sustenance of cancer, although its role in cancer remains controversial and poorly understood [8]). In the present work we found that the synthesis of IL-10 had a different behavior in the animals previously infected with *H. nana*. The highest IL-10 levels were found on the group that only received DMBA, which coincides with previous reports [1]; while the group administered with DMBA and infected with *H. nana* presented a significant reduction of this cytokine, a result similar to that reported by Reardon et al., using a colitis model and infecting mice with *Hymenolepis diminuta* [48]). The decrease of this cytokine is radically important since IL-10 is responsible for a negative regulation in the expression of MHC-I, MHC-II, co-stimulatory and adhesion molecules, which complicates the antigenic presentation favoring the development of tumor cells [49,54]; in addition, the expression of this cytokine has been associated with a state of generalized immunosuppression, which predisposes to a poor prognosis in the resolution of cervical dysplasia [40]. It is complicated that decreased IL-10 by itself represents a control mechanism for malignant cells; however, in the right context it could represent an advantage to limit the invasiveness of cancer. On the other hand, the administration of DMBA induced higher concentrations of IFNγ than the controls; this finding coincides with that reported in different experimental models where this chemical agent is used to generate cancer [1]. The importance of IFNγ in the control of tumors has been documented *in vivo* and *in vitro* observations, even in the model of skin papillomas induced by DMBA [30]. Although it is known that IFNγ and IL-5 are expressed in Th1 and Th2 inflammatory responses respectively, which often work in opposite ways, the former is a powerful mediator of the cellular response and latter acts as a humoral activator of effectors cells, and the combination of high serum concentrations of these cytokines has been reported as a factor that delays the development of tumors [41], phenomenon that corroborates our observations in this experimental protocol. Nevertheless, the specific effect of IFNγ in cancer has not been fully established and is still a source of debate, since it has been documented that it could have a dual effect, on one hand activating cytotoxic cells and limiting the development of tumors, and on the other hand participating in protumor mechanisms, when the stimulation of IFNγ is prolonged [56].

In order to explore the mechanism involved in the reduction of tumors in the group of animals previously infected with *H. nana*, serum concentrations of malonaldehyde were determined, a molecule that refers to lipid oxidation and serves as a marker of oxidative stress. In relation to this determination, two findings are important, the lowest concentrations were found in the group in which DMBA was administered, and this data can be explained by two previous reports; Abdalla and collaborators reported that the application of DMBA reduces the number of macrophages with M1 profile, cells essential for phagocytosis and lysis by generation of reactive oxygen species (ROS) [1], also this same group of animals presented the highest levels of IL-10, which is associated with decreased ROS [6,31]. On the other hand, the group with previous infection with the helminth and the administration of DMBA showed the highest levels of malondialdehyde, in agreement with what was previously documented by Niwa and Miyazato, who describe that mice infected with *H. nana* significantly increased ROS production. The authors also described that eosinophils present in tissues are the main source of these radicals [44]. With the above information, we directed the research towards eosinophils in animals infected with *H. nana* and subsequently treated with DMBA.

The administration of DMBA on the skin of mice induces the formation of papillomas, which are benign tumor lesions, however, this application also causes the malignization of the lesions until they become highly invasive carcinomas [50]. The transition from the benign tumor to the neoplasm is a slow process that involves a series of progressive changes in both the cells and the architecture of the tissues; in the case of experimental models such as clinical cases induced by human papillomavirus infection, variations in the basement membrane and keratinization are started until complete differentiation is reached, including cell multinucleation, as occurs in head and neck carcinomas

[25]. The results observed in this study are in agreement with data previously described, since the group of animals in which cancer was induced presented cellular alterations characteristic of papillomatous lesions, also signs of progression towards carcinoma were observed as the morphological alteration of the basement membrane and intense desmoplasic reaction [13]. In the opposite way, these signs of malignancy were not observed in the few and small tumors found in the group of mice infected previously with *H. nana*. Besides, an important and different aspect of the inflammatory infiltrate in the lesions were observed; previous reports describe that papillomas are commonly surrounded by a lymphoplasmacytic infiltrate [25]), in contrast, we found that tumors were surrounded by eosinophils and neutrophils in both groups receiving DMBA. This difference could be due to the fact that the lesions analyzed correspond to the first cancerous stages, but despite that, animals that had the highest number of eosinophils and neutrophils had a better resolution of the lesions. The presence of neutrophils is controversial because despite being a phagocyte with a repertory of cytotoxic molecules that could lyse tumor cells [19], there are multiple reports that associate the presence of these cells with the growth of benign and malignant tumors [19,33,37,57], but the specific role of these cells in the development of cancer is unknown. The presence of eosinophils could explain in part the control of cancer in group D; multiple studies have documented the presence of these cells surrounding or infiltrating tumors, improving the prognosis of cancer development, such as colon [16]), squamous cell carcinoma in the oral cavity [14], carcinoma of squamous cells in esophagus [26], nasopharyngeal carcinoma [18], pulmonary adenocarcinoma [11] and prostate cancer [34]. It has been suggested that degranulation on neoplasic cells would have a cytotoxic effect [10],Goetzl et al., suggested that the activity of eosinophils on tumors could be generic or specific depending on the previous activation of these cells [21]. However, the specific role of these cells and cancer remains under exploration.

All the above information supports the proposal that in addition to the Th1 response that is usually induced in response to cancer, a Th2 response plays an important role in the control of the development of neoplasms and increases the knowledge about the immune response generated by helminth parasites and tumorigenesis. However, in this work we emphasize that the immune response generated by the infection of a helminth parasite, activates cytotoxic mechanisms capable of limiting the growth and dispersion of tumors. The probability that one or several molecules derived from parasitic helminths are similar to those expressed by malignant cells is one of the current objectives of our research group.

The development of cancer is product of multiple causes, and its control in the same way involves a series of equally complex mechanisms. The knowledge of the relationship that this pathology has with other infectious diseases could give us the opportunity to propose new control mechanisms.

Funding source

This work was partially supported by Grant IN224814 from PAPIIT-UNAM, Mexico.

Declaration of Competing Interest

None

Acknowledgments

We thank Guillermina Garcia Avila for the assistance with the graphical abstract present on this paper, Ricardo Vargas Orozco for the professional care of the animals used in this work and Edith Mendoza Tenorio for her support in the determination of MDA.

References

[1] D.R. Abdalla, A.A. Aleixo, E.F. Murta, M.A. Michelin, Innate immune response adaptation in mice subjected to administration of DMBA and physical activity, Oncol. Lett. 7 (2014) 886–890.
[2] A. Ajami, A. Rafiei, Cytokine production in Hymenolepis nana infection, Iran J. Immunol. 4 (2007) 236–240.
[3] B. Amare, J. Ali, B. Moges, G. Yismaw, Y. Belyhun, S. Gebretsadik, D. Woldeyohannes, K. Tafess, E. Abate, M. Endris, D. Tegabu, A. Mulu, F. Ota, B. Fantahun, A. Kassu, Nutritional status, intestinal parasite infection and allergy among school children in northwest Ethiopia, BMC Pediatr 13 (2013) 7.
[4] S. Amu, S.P. Saunders, M. Kronenberg, N.E. Mangan, A. Atzberger, P.G. Fallon, Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model, J. Allergy Clin. Immunol. 125 (1114–1124) (2010) e1118.
[5] G. Avila, M. Benitez, L. Aguilar-Vega, A. Flisser, Kinetics of Taenia solium antibodies and antigens in experimental taeniosis, Parasitol. Res. 89 (2003) 284–289.
[6] K. Batcioglu, A.B. Uyumlu, B. Satilmis, B. Yildirim, N. Yucel, H. Demirtas, R. Onkal, R.M. Guzel, M.B. Djamgoz, Oxidative stress in the in vivo DMBA rat model of breast cancer: suppression by a voltage-gated sodium channel inhibitor (RS100642), Basic Clin. Pharmacol. Toxicol. 111 (2012) 137–141.
[7] A.K. Berntzen, M. Voge, In vitro hatching of oncospheres of four hymenolepidid cestodes, J. Parasitol. 51 (1965) 235–242.
[8] F.C.B. Berti, A.P.L. Pereira, G.C.M. Cebinelli, K.P. Trugilo, K. Brajao de Oliveira, The role of interleukin 10 in human papilloma virus infection and progression to cervical carcinoma, Cytokine Growth Factor Rev. 34 (2017) 1–13.
[9] V. Bordignon, E.G. Di Domenico, E. Trento, G. D'Agosto, I. Cavallo, M. Pontone, F. Pimpinelli, L. Mariani, F. Ensoli, How human papillomavirus replication and immune evasion strategies take advantage of the host DNA damage repair machinery, Viruses (2017) 9.
[10] R.A. Caruso, A. Parisi, E. Quattrocchi, M. Scardigno, G. Branca, C. Parisi, R. Luciano, D. Paparo, F. Fedele, Ultrastructural descriptions of heterotypic aggregation between eosinophils and tumor cells in human gastric carcinomas, Ultrastruct. Pathol. 35 (2011) 145–149.
[11] R. Costello, T. O'Callaghan, G. Sebahoun, Eosinophils and antitumour response, Rev. Med. Interne. 26 (2005) 479–484.
[12] A. de Jong, M.I. van Poelgeest, J.M. van der Hulst, J.W. Drijfhout, G.J. Fleuren, C.J. Melief, G. Kenter, R. Offringa, S.H. van der Burg, Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6, Cancer Res. 64 (2004) 5449–5455.
[13] M. Di Martino, I. Garcia Sanz, J.L. Munoz de Nova, C. Marin Campos, E. Martin-Perez, Comb sign in intestinal obstruction secondary to desmoplastic reaction due to an ileal neuroendocrine tumor, Rev. Esp. Enferm. Dig. 107 (2015) 697–698.
[14] R.G. Dorta, G. Landman, L.P. Kowalski, J.R. Lauris, M.R. Latorre, D.T. Oliveira, Tumour-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas, Histopathology 41 (2002) 152–157.
[15] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (2015) E359–386.
[16] M.J. Fernandez-Acenero, M. Galindo-Gallego, J. Sanz, A. Aljama, Prognostic influence of tumor-associated eosinophilic infiltrate in colorectal carcinoma, Cancer 88 (2000) 1544–1548.
[17] C.M. Fitzsimmons, F.H. Falcone, D.W. Dunne, Helminth allergens, parasite-specific IgE, and Its protective role in human immunity, Front Immunol 5 (2014) 61.
[18] M. Fujii, T. Yamashita, R. Ishiguro, M. Tashiro, K. Kameyama, Significance of epidermal growth factor receptor and tumor associated tissue eosinophilia in the prognosis of patients with nasopharyngeal carcinoma, Auris Nasus Larynx 29 (2002) 175–181.
[19] M. Gershkovitz, T. Fainsod-Levi, T. Zelter, R.V. Sionov, Z. Granot, TRPM2 modulates neutrophil attraction to murine tumor cells by regulating CXCL2 expression, Cancer Immunol. Immunother. (2018).
[20] N. Gevariya, M. Besancon, K. Robitaille, V. Picard, L. Diabate, A. Alesawi, P. Julien, Y. Fradet, A. Bergeron, V. Fradet, Omega-3 fatty acids decrease prostate cancer progression associated with an anti-tumor immune response in eugonadal and castrated mice, Prostate (2018).
[21] E.J. Goetzl, A.H. Tashjian Jr., R.H. Rubin, K.F. Austen, Production of a low molecular weight eosinophil polymorphonuclear leukocyte chemotactic factor by anaplastic squamous cell carcinomas of human lung, J. Clin. Invest. 61 (1978) 770–780.
[22] P. Gong, J. Zhang, L. Cao, Z. Nan, J. Li, J. Yang, H. Fang, H. Jiao, T. Jiang, L. Su, X. Zhang, Identification and characterization of myeloma-associated antigens in Trichinella spiralis, Exp. Parasitol. 127 (2011) 784–788.
[23] H.J. Gould, B.J. Sutton, A.J. Beavil, R.L. Beavil, N. McCloskey, H.A. Coker, D. Fear, L. Smurthwaite, The biology of IGE and the basis of allergic disease, Annu. Rev. Immunol. 21 (2003) 579–628.
[24] A. Gundelach, B. Henry, Cancer-Related Psychological Distress: A Concept Analysis, Clin. J. Oncol. Nurs. 20 (2016) 630–634.
[25] N. Husain, A. Neyaz, Human papillomavirus associated head and neck squamous cell carcinoma: controversies and new concepts, J. Oral. Biol. Craniofac Res. 7 (2017) 198–205.
[26] S. Ishibashi, Y. Ohashi, T. Suzuki, S. Miyazaki, T. Moriya, S. Satomi, H. Sasano, Tumor-associated tissue eosinophilia in human esophageal squamous cell carcinoma, Anticancer Res. 26 (2006) 1419–1424.
[27] E. Jensen-Jarolim, G. Achatz, M.C. Turner, S. Karagiannis, F. Legrand, M. Capron, M.L. Penichet, J.A. Rodriguez, A.G. Siccardi, L. Vangelista, A.B. Riemer, H. Gould,

AllergoOncology: the role of IgE-mediated allergy in cancer, Allergy 63 (2008) 1255–1266.
[28] D.H. Josephs, J.F. Spicer, C.J. Corrigan, H.J. Gould, S.N. Karagiannis, Epidemiological associations of allergy, IgE and cancer, Clin. Exp. Allergy 43 (2013) 1110–1123.
[29] J.P. Kinet, The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology, Annu. Rev. Immunol. 17 (1999) 931–972.
[30] J.H. Ko, B.G. Jung, Y.S. Park, B.J. Lee, Inhibitory effects of interferon-gamma plasmid DNA on DMBA-TPA induced mouse skin carcinogenesis, Cancer Gene. Ther. 18 (2011) 646–654.
[31] H. Kondo, S. Kira, T. Oniki, K. Gotoh, A. Fukui, I. Abe, Y. Ikebe, K. Kawano, S. Saito, K. Aoki, N. Okada, Y. Nagano, H. Akioka, T. Shinohara, K. Akiyoshi, T. Masaki, Y. Teshima, K. Yufu, M. Nakagawa, N. Takahashi, Interleukin-10 treatment attenuates sinus node dysfunction caused by streptozotocin-induced hyperglycemia in mice, Cardiovasc. Res. (2018).
[32] L.S. Leoh, T.R. Daniels-Wells, M.L. Penichet, IgE immunotherapy against cancer, Curr. Top. Microbiol. Immunol. 388 (2015) 109–149.
[33] Y. Li, D.L. Wheeler, W. Alters, L. Chaiswing, A.K. Verma, T.D. Oberley, Early epidermal destruction with subsequent epidermal hyperplasia is a unique feature of the papilloma-independent squamous cell carcinoma phenotype in PKCepsilon overexpressing transgenic mice, Toxicol. Pathol. 33 (2005) 684–694.
[34] S. Luna-More, P. Florez, A. Ayala, F. Diaz, A. Santos, Neutral and acid mucins and eosinophil and argyrophil crystalloids in carcinoma and atypical adenomatous hyperplasia of the prostate, Pathol. Res. Pract. 193 (1997) 291–298.
[35] M.E. Lund, B.A. O'Brien, A.T. Hutchinson, M.W. Robinson, A.M. Simpson, J.P. Dalton, S. Donnelly, Secreted proteins from the helminth Fasciola hepatica inhibit the initiation of autoreactive T cell responses and prevent diabetes in the NOD mouse, PLoS One 9 (2014) e86289.
[36] W. Ma, C.J. Melief, S.H. van der Burg, Control of immune escaped human papilloma virus is regained after therapeutic vaccination, Curr Opin Virol 23 (2017) 16–22.
[37] Y. Mao, Q. Feng, P. Zheng, L. Yang, T. Liu, Y. Xu, D. Zhu, W. Chang, M. Ji, L. Ren, Y. Wei, G. He, J. Xu, Low tumor purity is associated with poor prognosis, heavy mutation burden, and intense immune phenotype in colon cancer, Cancer Manag. Res. 10 (2018) 3569–3577.
[38] L. Mendonca, R. Dos Santos Ferreira, R. de Cassia Avellaneda Guimaraes, A.P. de Castro, O.L. Franco, R. Matias, C.M.E. Carvalho, The Complex puzzle of interactions among functional food, gut microbiota, and colorectal cancer, Front. Oncol. 8 (2018) 325.
[39] D.S. Michaud, S.M. Langevin, M. Eliot, H.H. Nelson, M.D. McClean, B.C. Christensen, C.J. Marsit, K.T. Kelsey, Allergies and risk of head and neck cancer, Cancer Causes Control 23 (2012) 1317–1322.
[40] R. Mindiola, D. Caulejas, J. Nunez-Troconis, M. Araujo, M. Delgado, J. Mosquera, Increased number of IL-2, IL-2 receptor and IL-10 positive cells in premalignant lesions of the cervix, Invest. Clin. 49 (2008) 533–545.
[41] J. Miyaguchi, K. Shiga, K. Ogawa, F. Suzuki, K. Katagiri, D. Saito, A. Ikeda, A. Horii, M. Watanabe, S. Igimi, Treatment with lactobacillus retards the tumor growth of head and neck squamous cell carcinoma cells inoculated in mice, Tohoku J. Exp. Med. 245 (2018) 269–275.
[42] C.C. Motran, L. Silvane, L.S. Chiapello, M.G. Theumer, L.F. Ambrosio, X. Volpini, D.P. Celias, L. Cervi, Helminth infections: recognition and modulation of the immune response by innate immune cells, Front Immunol. 9 (2018) 664.
[43] K. Mukai, M. Tsai, P. Starkl, T. Marichal, S.J. Galli, IgE and mast cells in host defense against parasites and venoms, Semin. Immunopathol. 38 (2016) 581–603.
[44] A. Niwa, T. Miyazato, Reactive oxygen intermediates from eosinophils in mice infected with Hymenolepis nana, Parasite Immunol. 18 (1996) 285–295.
[45] V. Noya, S. Bay, M.F. Festari, E.P. Garcia, E. Rodriguez, C. Chiale, C. Ganneau, F. Baleux, S. Astrada, M. Bollati-Fogolin, E. Osinaga, T. Freire, Mucin-like peptides from Echinococcus granulosus induce antitumor activity, Int. J. Oncol. 43 (2013) 775–784.
[46] P. Ramanathan, H. Dhandapani, H. Jayakumar, S. Ganeshrajah, R. Thangarajan, Dendritic cells primed with HPV positive cervical tumor lysate are superior to unprimed DCs in migratory capacity and induce a potent Th1 response, Hum. Immunol. 75 (2014) 1216–1224.
[47] E. Ramos-Martinez, M.R. Lopez-Vancell, J.C. Fernandez de Cordova-Aguirre, J. Rojas-Serrano, A. Chavarria, A. Velasco-Medina, G. Velazquez-Samano, Reduction of respiratory infections in asthma patients supplemented with vitamin D is related to increased serum IL-10 and IFNgamma levels and cathelicidin expression, Cytokine 108 (2018) 239–246.
[48] C. Reardon, A. Sanchez, C.M. Hogaboam, D.M. McKay, Tapeworm infection reduces epithelial ion transport abnormalities in murine dextran sulfate sodium-induced colitis, Infect. Immun. 69 (2001) 4417–4423.
[49] R. Sabat, G. Grutz, K. Warszawska, S. Kirsch, E. Witte, K. Wolk, J. Geginat, Biology of interleukin-10, Cytokine Growth Factor Rev. 21 (2010) 331–344.
[50] M. Schwarz, P.A. Munzel, A. Braeuning, Non-melanoma skin cancer in mouse and man, Arch. Toxicol. 87 (2013) 783–798.
[51] M. Serrano, A.W. Lin, M.E. McCurrach, D. Beach, S.W. Lowe, Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a, Cell 88 (1997) 593–602.
[52] N. Taghipour, H.A. Aghdaei, A. Haghighi, N. Mossafa, S.J. Tabaei, M. Rostami-Nejad, Potential treatment of inflammatory bowel disease: a review of helminths therapy, Gastroenterol Hepatol. Bed. Bench 7 (2014) 9–16.
[53] P. Tiwari, K.P. Gupta, Modulation of miR-203 and its regulators as a function of time during the development of 7, 12 dimethylbenz [a] anthracene induced mouse skin tumors in presence or absence of the antitumor agents, Toxicol. Appl. Pharmacol. 278 (2014) 148–158.
[54] K. Torres-Poveda, M. Bahena-Roman, C. Madrid-Gonzalez, A.I. Burguete-Garcia, V.H. Bermudez-Morales, O. Peralta-Zaragoza, V. Madrid-Marina, Role of IL-10 and TGF-beta1 in local immunosuppression in HPV-associated cervical neoplasia, World J. Clin. Oncol. 5 (2014) 753–763.
[55] R.A. Webb, T. Hoque, S. Dimas, Expulsion of the gastrointestinal cestode, Hymenolepis diminuta by tolerant rats: evidence for mediation by a Th2 type immune enhanced goblet cell hyperplasia, increased mucin production and secretion, Parasite. Immunol. 29 (2007) 11–21.
[56] M.R. Zaidi, The interferon-gamma paradox in cancer, J. Interferon. Cytokine Res. 39 (2019) 30–38.
[57] L. Zhao, C.W. Li, P. Jin, C.L. Ng, Z.B. Lin, Y.Y. Li, T.Y. Li, B.F. Petersson, L. Shi, Y. Wang de, Histopathological features of sinonasal inverted papillomas in chinese patients, Laryngoscope 126 (2016) E141–147.