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ORIGINAL PAPER

Histopathological and immunophenotyping studies on normal and sarcoma 180-bearing mice treated with a complex homeopathic medication

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Canova **is a homeopathic complex medicine, used as an immune modulator. We studied its effects in normal and sarcoma 180-bearing mice. Three control groups were also evaluated. The mice were examined at daily intervals and the tumours observed histologically. Peripheral blood was analysed by flow cytometry.**
A delay in the development, and a reduction in size of the tumours, and increased infiltration by lymphoid cells, granulation tissue, and fibrosis surrounding the tumour were observed with active treatment compared to control. All animals from the treated group survived, 30% of control groups died. In 30% of treated animals, a total regression of the tumour was confirmed using light microscopy, no regression was found in the control groups. Treatment with *Canova* increased total numbers of leukocytes and lymphocytes. Among lymphocytes, TCD4, increased in normal-treated group and B and NK cells in S180-treated groups. The results reflect enhanced immune response of the host after treatment with *Canova*. *Homeopathy* (2005) **94,** 26–32.

Keywords: immunotherapy; sarcoma 180; immunophenotyping; *Canova*

Introduction

Cancer results from tumour initiation and promotion, ultimately leading to tumour metastasis.[1] It is the second most frequent cause of mortality in humans.[2] The efficacy of cancer treatment is increasing, albeit slowly.[3] Advances in understanding fundamental mechanisms of regulation of the immune system are leading to the development of new strategies for promoting more effective immune responses against tumours.[4] Tumour immunotherapy aims at activation of the body's immune system against an existing tumour.[5] Therapy directed at enhancing immunity to favour a particular immunological response against malignant cells is a form of immunotherapy referred to as 'biological response modification'.[6]

The immune system consists of cells and molecules with specialized roles in defending the body mainly against infection and neoplasia, and maintaining homeostasis. It is controlled by differentiated T and B cell clones and activated macrophages. There is now evidence that T cell responses occur in several human cancers,[7] and that Natural Killer (NK) cell activity contributes to anti-tumour efficacy.[8] One of the functions of macrophages is to provide a defence mechanism against tumour cells. Macrophage activation can play a role in immunotherapeutic approaches to the treatment of cancer.[9]

Sarcoma 180 (S-180), also known as Crocker's tumour, grows rapidly in most (>90%) animals in

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which is it inoculated, but regresses in 8–10%. This regression rate can be increased by treatment with certain chemicals or biological products.[10]

Canova (CA) is a complex homeopathic medicine produced in Brazil. In spite of clinical reports showing successful results when it was used in diseases where the immune system was depressed,[11] accurate scientific studies are lacking. However, it seems to enhance the individual's immunologic response in several pathological states. It is neither toxic, nor mutagenic.[12] *In vitro* and *in vivo* studies demonstrate that immune response activation occurs at both cellular and molecular levels, macrophages exhibit morphological and molecular changes indicating activation, and Tumour Necrosis Factor-α (TNF-α) production decreases significantly.[13]

We report an investigation of its effects on immunological responses to a tumour. This includes observation of lymphoid infiltration, peritumoural fibrosis, growth and tumour size, leukocyte immunophenotyping in peripheral blood of normal mice and sarcoma 180 (S180)-bearing mice.

Materials and methods

Canova

'Canova do Brasil', a Brazilian company, holds the international patent to this medicine (www.canovadobrasil.com.br). *Canova* is produced in drops, inhalant and intravenous forms, and sold only by authorized pharmacies and laboratories. *Canova* is standardized and authorized by competent agencies for medicinal application. Mother tinctures are purchased from suppliers authorized by the Brazilian Health Ministry. The final product, *Canova*, contains *Aconitum napellus* (Ranunculaceae) 11dH, *Bryonia alba* (Curcubitaceae) 18dH, *Thuja ocidentalis* (Cupresaceae) 19dH, *Arsenicum album* (Arsenious trioxide) 19dH and *Lachesis muta* (Viperidae) 18dH and less than 1% alcohol all in distilled water. It is an aqueous, colourless and odourless solution. Experiments were performed with commercial *Canova* donated by Canova do Brasil. The final two decimal dilutions were performed in our laboratory.

Animal and *Canova* treatment

Fifty five male albino Swiss mice from the Animal Facilities at Universidade Federal do Paraná (UFPR), weighing between 30 and 35 g, and 3–4 months old, were selected and kept in a 12-h light/dark cycle. They were fed with standard food and water *ad libitum*. All recommendations of the National Law (No 6.638, November, 5th 1979) for scientific management of animals were respected. They were divided into 5 groups of 11 animals each, and treated as follows:

normal group (N)—normal mice with neither tumour nor treatment;

treated normal group (NT)—normal mice treated with *Canova*;

sarcoma group (S)—mice with tumour no treatment;

treated sarcoma group (ST)—mice with tumour, treated with *Canova*;

sarcoma treatment control group (Stc)—mice with tumour treated with water–ethanol solution (0.01% alcohol in distilled water).

Treated mice received, at daily intervals, a subcutaneous injection of 7 µl/g commercial *Canova* for 19 days, starting 24 h after tumour inoculation. The injections were performed alternately in the left and right side posterior-dorsal region, away from the site of tumour inoculation. A sucussion (shaking) process was performed on the medication and water–ethanol control just before administration.

Tumour inoculation

The ascitic form of sarcoma-180 (S-180) was maintained in mice by intraperitoneal inoculation. Seven-day-old S180 ascitic cells (5×10^6 cells) were transplanted subcutaneously into the cervico-dorsal region of the mice according to the procedure of Shirai *et al*.[14] Tumour growth was observed for 21 days.

Blood collection

All the animals were killed on the 21st day after tumour inoculation. The mice were anaesthetized with ether and blood collected by cardiac puncture using a sterile syringe and stored in a tube containing EDTA K_3 (Vacuette K_3). The total number of white blood cells (WBC) was determined using a Cell Blood Automatic Counter STKS. For differential cell counting, blood smears were prepared and stained with May-Grünwald Giemsa (Pró-Cito, RS, Brazil) and observed by light microscopy. The remaining blood cells were used for flow cytometric analysis.

Tumour examination

Tumour volume analysis was performed with a pachymeter. It was measured in three dimensions and presence or absence of ulceration evaluated macroscopically.

The tumours were removed and fixed in a 10% neutral buffered formalin solution. After embedding in paraffin, 4 µm sections were prepared and stained with hematoxylin and eosin (HE) for microscopic observation. Sections from the periphery of the tumour were stained with Masson trichromic and argent impregnation, special histotechniques for type I collagen and type III collagen (reticular fibres). Tumour invasion and infiltration in tissues and organs was also analysed. Quantification of lymphocytes surrounding the tumour was based on the following scale: absent:0; few, isolated or grouped but not surrounding the tumour:1; surrounding tumour, forming a ring of variable thickness:2; surrounding tumour, forming a ring of constant thickness:3.

After special staining for collagen fibres (Masson trichromic), the slides were analysed under light

microscopy. Fibrosis was quantified by the scale: absent:0; non-continuous fibrosis:1; continuous fibrosis forming a ring of variable thickness:2; fibrosis forming a thick and continuous ring:3. We also counted the number of mitoses within 10 microscope fields with 40 × objective.

The scoring and analyses were conducted blind. The identification of tumour sections slides was covered and numbered by a person not otherwise involved in the experiment. The slides were evaluated by two persons. The photos of the sections were obtained by an Olympus BHS photomicroscope. We used ANOVA and or Kruskal–Wallis test to verify statistical differences within groups. When differences were found, we used Tukey's and or Gomes' test to verify these differences.

Cell labelling and flow cytometric analysis

Leukocytes were labelled using monoclonal antibodies (mAbs) anti-CD45 and CD11b. NK Lymphocytes were specifically marked using mAbs anti-DX5; B cells by anti-CD19; and T by anti-CD3. Subpopulations of T cells were distinguished by using mAbs anti-CD4 and CD8. A fluorescent anti-antibody was used. Antibodies are listed in Table 1.

Blood suspensions containing 0.5×10^6 WBC were incubated with mAbs for 30 min at 4 °C in the dark. The red blood cells were lysed using Optlyse C (Coulter-Immunotech, Marseille, FR). Multicolour flow cytometric analysis was performed using a COULTER EPICS-XL Flow Cytometer (Marseille, FR) equipped with an argon ion laser operated at 488 nm. Fluorescent emissions were logarithmically amplified and electronically compensated to eliminate overlapping spectral emission. All data were collected by listmode acquisition and analysed using Gateway (Coulter) software.

Immunophenotyping data were transformed as square roots and were analysed using one-way analysis of variance (ANOVA) with factorial diagram (2 × 2). The *T*-test was performed when the effects of interaction was significant. The level of significance was taken at $P<0.05$ and $P<0.01$.

Table 1 Monoclonal anti-bodies (mAbs) used for immunophenotyping cells from peripheral blood using two-colour flow cytometry

Antibodies	Clone
FITC[a]/PE Ig G_1/Ig G_1 (γ_1/γ_1)[c]	opticlone
FITC anti-mouse CD45 (Ly-5, LCA)	30-F11
FITC anti-mouse CD3 complex molecular	17A2
R-PE[b] anti-mouse CD4 (L3T4)	RM4-5
R-PE anti-mouse CD8a (Ly-2)	53-6.7
R-PE anti-mouse CD19	1D3
R-PE anti-mouse CD11b(α Mac-1, α_M integrin)	M1/70
R-PE anti-mouse Pan-cells NK	DX5

[a]Fluorescein-labelled mAb.
[b]R-Phycoerythrin-labelled mAb.
[c]Purchased from Coulter-Immunitech (Marseille, FR) and all other mAb were purchased from BD PharMingen (San Diego, CA).

Results

Tumour macroscopic observations

There was a significant delay in the time that to feel the tumour digitally ($P<0.01$); tumour size ($P<0.05$) was significantly less in the treated group (ST) compared with the S and Stc control groups (Figure 1A and B). Cutaneous ulceration was also less frequent in the treated group. There was a total regression of the tumour, confirmed by histopathologic examination, in 30% of animals in the *Canova*-treated group (ST). Those treated with *Canova* did not lose weight, whereas most of the mice from control groups, S and Stc, had a significant loss. All animals from ST group survived, 30% of those of the control groups (S and Stc) died during the experiment.

Histopathologic observations on tumours

A large portion of the central area of the tumours from the sarcoma group (S) and sarcoma treatment control (Stc) showed necrosis after 20 days, with some foci of calcification. Typical and atypical mitoses were seen in both groups. Some of the tumour cells formed by binuclear or multinuclear giant cells became swollen and had intensely eosinophilic cytoplasm. Isolated tumour cells were seen in contact with blood vessels. An important change in necrosis distribution was observed in tumours from *Canova*-treated animals: in

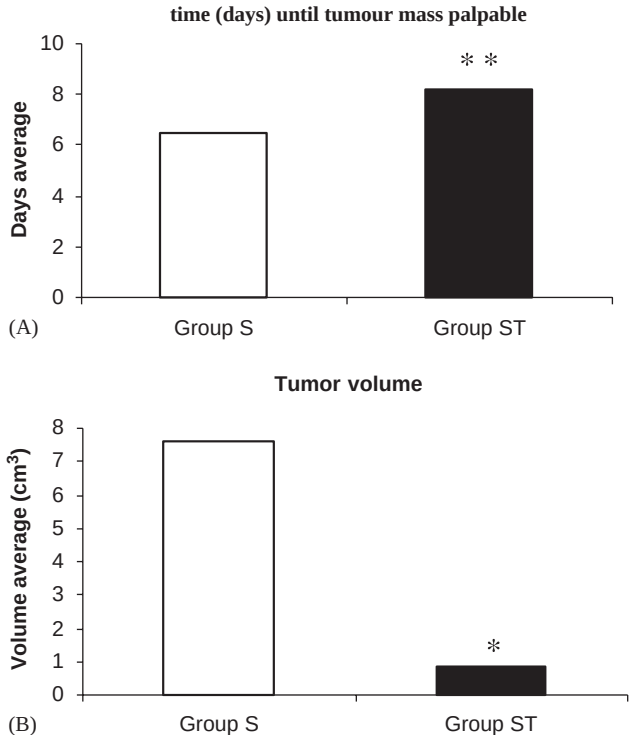


Figure 1. (A and B) Time in days until tumour palpable digitally. This was significantly different between control (S) and *Canova*-treated (ST) groups. (B) Tumour volume on 21st day diminished significantly in the *Canova*-treated group (ST), compared to the control group (S). ANOVA with factorial diagram (2x2) and *T*-test were performed ($^{*}P<0.05$ and $^{**}P<0.01$).

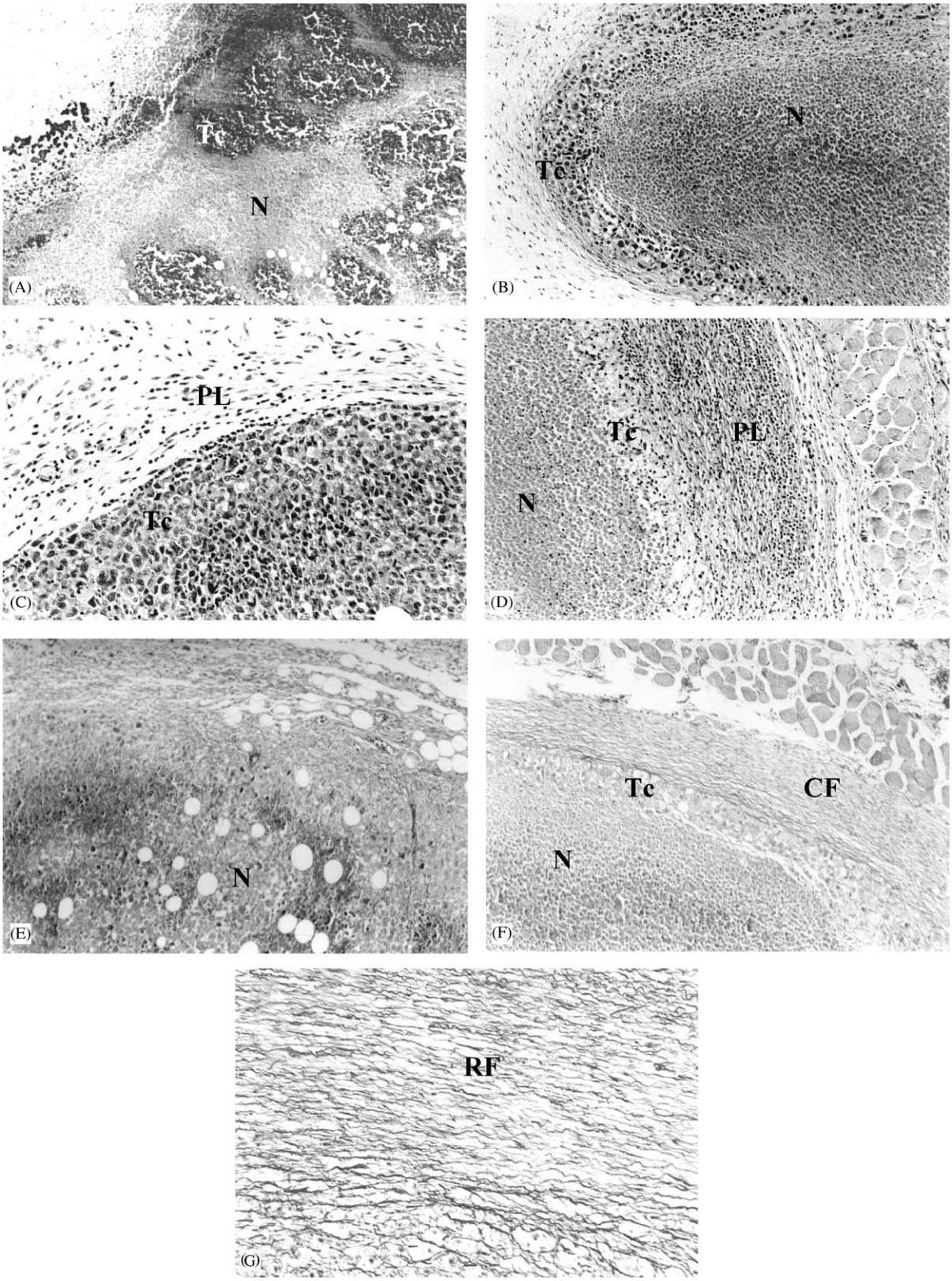


Figure 2. Microphotographs from sarcoma 180 control and sarcoma 180 treated with *Canova* medication. (A, C and E) show sarcoma 180 tumour control (S group), with islands of tumour cells (Tc) between multifocal areas of necrosis (N) and few peritumoural leukocytes (PL). (B, D, F and G) show sarcoma 180 treated with CA (ST group) with few viable neoplastic cells restricted to periphery of the tumour mass (Tc) around a central area of necrosis (N), a dense infiltrate of leukocytes surrounding the tumour mass (PL). Masson trichromic stain (F) shows dense type I collagen fibres (CF). Argent impregnation (G) shows a thick layer of type III collagen (reticular fibres—RF) surrounding the tumour, resulting in encapsulation. Final magnification of each photo: 100 $\times$.

Table 2 Summary of tumour data

	Palpable mass (d)	Final volume (cm^3)	Mitosis (mean/ field)	Peritumoural lymphocytes (scale 0–4)	Peritumoural fibrosis (scale 0–4)
S	6.5 (0.5)	7.6 (2.4)	11.9 (4.5)	1.5 (0.5)	0.6 (0.5)
ST	8.2 (1.1)**	0.9 (1.2)*	3.7 (2.7)**	2.3 (0.7)*	2.1 (0.6)*
Stc	6.6 (1.6)	5.4 (9.3)	9.5 (4.00)	1.3 (0.5)	1.0 (0.5)

The results are given as mean ± standard deviation ($^{*}P<0.05$) or 1% ($^{**}P<0.01$).

control groups there were several necrotic regions among isles of tumour cells (Figure 2A and E); but in the sarcoma-treated (ST) group necrosis was confined to a single central area (Figure 2B, D and F). The histopathologic studies confirmed total tumour regression in 30% of animals in the ST group. In this group, the histopathologic analysis revealed only a few residual neoplastic cells between a large central necrotic area and peripheral fibrous tissue.

The infiltration of inflammatory cells around the tumour was more intense in the ST group. There was a significant difference ($P<0.05$) comparing the peritumoural leukocyte infiltrates of the ST group (Figure 2D) with those of control groups (S and Stc) (Figure 2C). The tumours from mice treated with *Canova* were surrounded by a dense ring of eukocytes (Figure 2D). The data are summarized on Table 2.

There was a significant difference ($P<0.05$) in peritumoural fibrosis of the ST group when compared with others groups. In treated mice fibrosis was evident around the tumour. There was a dense layer of type I collagen (Figure 2F) and a thick net of type III collagen (reticular fibres) (Figure 2G). These fibres formed a capsule-like structure surrounding the tumour, resulting in a delimitation of the invasive tumour mass.

Immunophenotyping

The normal animals (N group) had a mean 6.7×10^3 leukocytes/μl: 77% lymphocytes, 16% granulocytes, and 7% monocytes. Among the lymphocytes, 44% B cells, 22% T CD4, 10% T CD8, and 0.71% NK were found. In the treated normal group (NT), treatment with *Canova* appears to induce an up-regulation in total leukocyte counts and an increase in the number of lymphocytes TCD4 in peripheral blood (Figure 3). The sarcoma bearing animals (group S) showed up-regulation in granulocyte number and down-regulation of all the other leukocytes, including all lymphocyte subgroups examined (Figure 4). In the treated sarcoma group (ST), treatment with *Canova* induced an up-regulation in total leukocyte count. The number of lymphocytes and monocytes were close to normal, apparently neutralizing the presence of the tumour (Figure 4). There was a significant increase in NK cell numbers (Figure 5).

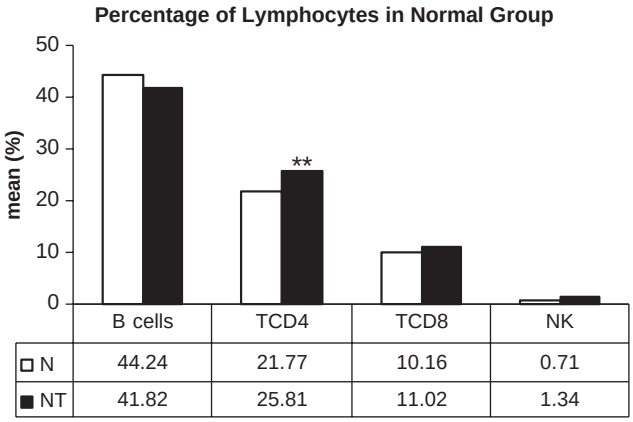
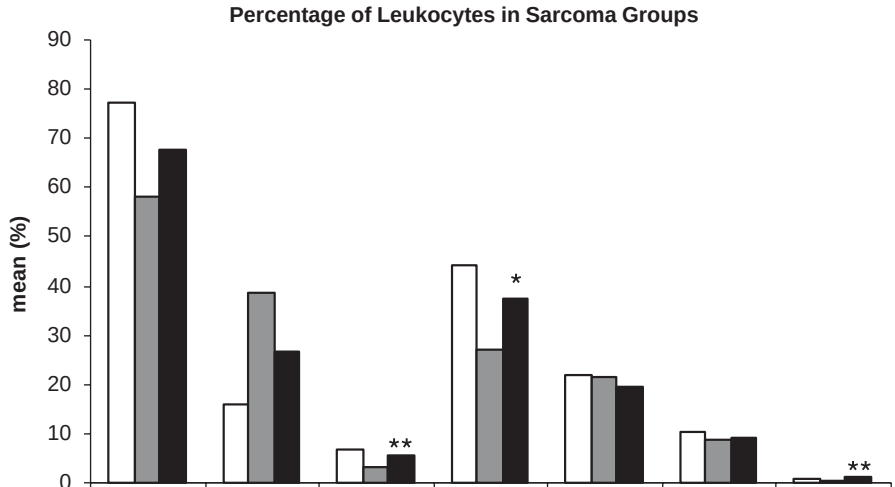


Figure 3. Effect of treatment with CA on lymphocyte percentages in peripheral blood of non-tumour bearing animals treated with Canova and control (flow cytometer). ANOVA with factorial diagram (2x2) and *T*-test were performed ($^{**}P<0.01$).

Discussion

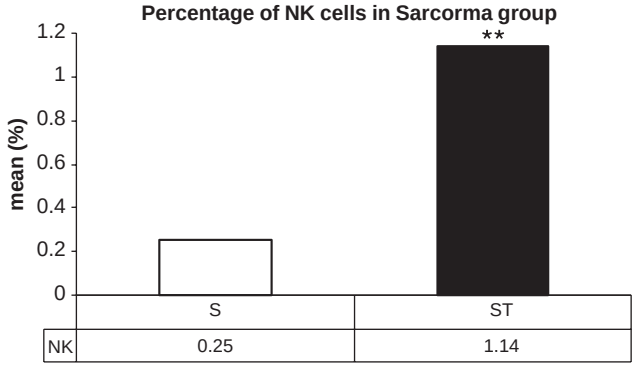
Clinical and pathological evidence indicate that some tumours stimulate immune responses. We studied *Canova* to evaluate it as a new cancer therapy.[3] The immune system plays a role in prevention of development and in control of tumour growth.[6] Among the cells engaged in the immune response, macrophages act as coordinators, they present antigens and secrete cytokines, such as TNF-α and interleukins.[15] Both actions are important for the humoral and cellular responses. Therefore, any substance that enhances these processes may be used, in addition to conventional therapy for the control of cancer.

The tumours in the *Canova*-treated group had a lower rate of growth with a delay in the appearance of a palpable mass. The reduced production of TNF-α in the *Canova* group[13] may explain why the treated animals group did not lose weight. The tumour size was significantly smaller and in 30% of the animals there was total tumour regression after 20 days treatment. The major distinction between malignant and benign tumours is the invasiveness of the former and their ability to metastasize to other regions of the body.[1] Histological observation indicated an important increase in the number of lymphocytes around the tumour as well as a larger ring of fibrosis in treated mice. These findings suggest that the immune system was stimulated and providing an increased response against the tumour. This was demonstrated by the regression of



	Ly	Gran	Mo	B	TCD4	TCD8	NK
N	77.42	15.81	6.77	44.24	21.77	10.16	0.71
S	58.12	38.75	3.13	27.19	21.48	8.8	0.25
ST	67.65	26.81	5.54	37.49	19.51	9.24	1.14

Figure 4. Graph and table showing the effect of treatment on leukocytes and the lymphocyte percentage in peripheral blood of sarcoma (S) and sarcoma-treated (ST) groups (flow cytometer). Leukocyte parameters of normal mice (N) are included to show that sarcoma alters the normal parameters and the treatment with CA apparently neutralizes the presence of the tumour, reaching values close to those of the normal standards. ANOVA with factorial diagram (2x2) and *T*-test were performed ($^{*}P<0.05$ and $^{**}P<0.01$).



	S	ST
NK	0.25	1.14

Figure 5. (detail) of Figure 4. Effect of treatment with CA on the NK lymphocyte percentage in peripheral blood of sarcoma (S) and sarcoma-treated (ST) groups (flow cytometer). Sarcoma 180 decreased the ratio of NK cell/leukocyte, CA treatment increased NK cells. ANOVA with factorial diagram (2x2) and *T*-test were performed ($^{**}P<0.01$).

the tumour or by its isolation through encapsulation by fibrous tissue.

An interaction between tumour cells and the extracellular matrix is critical during the invasive process and may be influenced by endogenous mediators, such as TNF-α, which can increase the expression of integrins to enhance cell adhesion and migration.[10,16] Collagen fibres or dense reticular fibres may have a role in limiting invasion by malignant cells.[10,15] Invasive growth of primary tumours and metastases depends on the destruction and remodelling of the stromal architecture. A body of evidence suggests that the production of metalloproteinases (MMP) from macrophages is stimulated by TNF-α, the presence of these molecules inhibit collagen production.[17–19] The ring of collagen and dense reticular fibres layer surrounding the tumour mass, limiting the invasiveness of sarcoma-180 cells, may be explained by the decrease in TNF-α production in *Canova*-treated group.[13]

The S180-bearing mice (S group) demonstrated an up-regulation in granulocytes and down-regulation in B lymphocytes. According to the literature, the colony-stimulating factor (CSF) produced by tumour cells in S180-bearing mice, increases the number of circulating peripheral polymorphonuclear cells (PMN). Granulopoiesis in S180-bearing mice has also been found.[20,21] Our results demonstrate that the S180 tumour significantly modifies these leukocyte parameters. *Canova* evidenced a tendency to normalize leukocyte parameters, mostly those of lymphocytes and monocytes. These cells play a central role in the defence of the host against the tumour.[9] Treatment of normal mice with *Canova* (NT group) induced an up-regulation in total leukocyte counts and all the investigated lymphocytes subtypes, compared to normal control (N), except for the NK percentage which was upregulated. NK lymphocytes are important because they can kill some cancer cells without either prior sensitization or requirement for MHC restriction.[2] They recognize cancer cells that express or not at least one of the MHC class I alleles of the host.

Comparing the results found in S180-bearing mice treated with *Canova* (ST group) with the S180-bearing mice without treatment (S group), we suggest that *Canova* probably acts through leukocyte regulations.

Canova appears not only to contain the tumour, but also normalizes the leukocyte proportions, compensating the effects of the disease.

The immune system plays a role in prevention of the development and control of tumour growth. Our findings suggest that in this model the immune system was stimulated by *Canova* and could be providing a better response against the tumour. This response was demonstrated by the regression of the tumour, by its isolation through an encapsulation by fibrous tissue, and immunophenotyping results. There is a growing consensus that anti-cancer treatments must be based on exploiting the host's own anti-tumour defense mechanisms.[22] Medications like *Canova*, which provide immunological therapy with no side effects, based on regulating the anti-tumour defense of the host, may be a useful adjuvant in cancer therapies.

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References

1 Bharti AC, Aggarwal BB. Nuclear factor-kappa B and cancer: its role in prevention and therapy. *Biochemical Pharmacology* 2002; **64**: 883–888.
2 Schreiber H. Tumour immunology. in: Paul W editor. *Fundamental Immunology*. Philadelphia: Lippincott-Raven Publishers, 1999. p. 1237–1270.
3 Desoize B. Stromal reaction and tumour growth. *Clinical Reviews in Oncology Hematology* 2004; **49**: 173–176.
4 Rosemberg SA. Principles and applications of biologic therapy. in: De Vita VT, Hellmann S, Rosemberg SA editors. *Cancer: Principles & Practice of Oncology*. Philadelphia: JB Lippincott, 1993. p. 276–292.
5 Gunzer M, Jänich S, Varga G, Grabbe S. Dendritic cells and tumour immunity. *Seminars in Immunology* 2001; **13**: 291–302.
6 Boura P, Kountouras J, Lygidakis NJ. Tumour immunity and immunotherapy. *Hepato-Gastroenterology* 2001; **48**: 1040–1044.
7 Ballow M, Nelson R. Immunopharmacology—immunomodulation and immunotherapy. *JAMA* 1997; **278**(22): 2008–2017.
8 Melo PS, Justo GZ, Durán N, Haun M. Natural killer cell activity and anti-tumour effects of dehydrocrotonin and its synthetic derivatives. *European Journal of Pharmacology* 2004; **487**: 47–54.
9 Klimp AH, Vries EGE, Scherphof GL, Daemen T. A potential role of macrophage activation in the treatment of cancer. *Clinical Reviews in Oncology Hematology* 2002; **44**(2): 143–161.
10 Assef MLM, Carneiro-Leão AM, Moretão MP, et al. Histological and immunohistochemical evaluation of Sarcoma 180 in mice after treatment with an α-D-glucan from the lichen *Ramalina celastri*. *Brazilian Journal of Morphological Science* 2002; **19**(2): 49–54.
11 Sasaki MGM, Mariano FC, Gurgel L, Probst S. Estudo Clínico randomizado placebo controlado apara avaliar a eficácia e segurança do Método Canova na terapêutica de pacientes portadores de HIV/AIDS em uso de anti-retrovirais. *Brazilian Journal of Infectious Disease* 2001; **5**(Supplement 1): 58.
12 Seligmann IC, Lima PDL, Cardoso PCS, et al. The anticancer homeopathic composite "Canova Method" is not genotoxic for human lymphocytes *in vitro*. *Genetics and Molecular Research* 2003; **2**(2): 223–228.
13 Piemonte MR, Buchi DF. Analysis of IL-2, IFN-γ and TNF-α production, $\alpha_5\beta_1$ integrins and actin filaments distribution in intraperitoneal mouse macrophages treated with homeopathic medicament. *Journal of Submicroscopy Cytology and Pathology* 2002; **3**: 255–263.
14 Shirai M, Izumi H, Yamagami T. Experimental transplantation models of mouse Sarcoma 180 mice for evaluation of anti-tumour drugs. *Journal of Veterinarian Medical Sciences* 1991; **53**(4): 707–713.
15 Abdullah H, Greenman J, Pimenidou A, Topping KP, Monson J. The role of monocytes and natural killer cells in mediating antibody-dependent lysis of colorectal tumour cells. *Cancer Immunology Immunotherapy* 1999; **48**: 517–524.
16 Kawzshima A, Kawahara E, Tokuda R, Nakanishi I. Tumour necrosis factor-alpha provokes upregulation of alfa2 beta1 and alph5 beta1 integrins, and cell migration on OST osteosarcoma cells. *Cell Biology International* 2001; **25**: 319–329.
17 Galis SZ, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis—the good, the bad and the ugly. *Circulation Research* 2002; **90**(3): 251–262.
18 Atamas SP. Complex cytokine regulation of tissue fibrosis. *Life Sciences* 2002; **72**(6): 631–643.
19 Birkedal-Hanse H, Moore WGI, Bodden MK, et al. Matrix metalloproteinases: a review. *Critical Reviews in Oral Biology and Medicine* 1993; **4**(2): 197–250.
20 Chasseing NA, Medina PD, Rumi LS. Leukocyte alterations in Sarcoma 180-bearing mice. *Medicina (Bueno Aires)* 1988; **48**: 172–178.
21 Okawa Y, Murata Y, Kobayashi M, Suzuki ME, Suzuki S. Augmentation of host resistance to *Candida albicans* infection in ascite tumour-bearing mice. *Microbiology and Immunology* 1992; **36**(5): 517–521.
22 Ehrke MJ. Immunomodulation in cancer therapeutics. *International Immunopharmacology* 2003; **3**: 1105–1119.