Cancer Immunol Immunother (2002) 50: 597–603
DOI 10.1007/s00262-001-0238-2

ORIGINAL ARTICLE

**Gabriel A. Rabinovich · Natalia Rubinstein
Pablo Matar · Viviana Rozados · Silvia Gervasoni
O. Graciela Scharovsky**

The antimetastatic effect of a single low dose of cyclophosphamide involves modulation of galectin-1 and Bcl-2 expression

Received: 17 August 2001 / Accepted: 29 September 2001 / Published online: 22 November 2001

Abstract We have demonstrated that a single low dose of cyclophosphamide has an antimetastatic effect on lymphoma (L-TACB)-bearing rats by modulating the host immune response. Galectin-1, a member of the galectin family with specificity for β-galactosides, has potent immunomodulatory properties by regulating cell-matrix interactions and T-cell apoptosis. Since galectin-1 is expressed by highly metastatic tumors, in the present study we investigated the participation of this β-galactoside-binding protein in cyclophosphamide-induced immunomodulation. Inbred "*e*" rats were s.c. challenged with L-TACB. After 14 days, half of the animals received an i.p. injection of cyclophosphamide (10 mg/kg), and on day 21 tumors and spleens were excised. Cell extracts were prepared and galectin-1 expression was determined by Western blot analysis and correlated with Bcl-2 expression levels and the DNA fragmentation profile. Expression of galectin-1 was significantly decreased in tumors from cyclophosphamide-treated rats compared to non-treated rats. The same effect was observed regarding expression of Bcl-2 by tumors. In contrast, expression of Bcl-2 was significantly higher in spleens from treated animals than in non-treated rats. This effect correlated with a decreased intensity in the pattern of DNA fragmentation of spleen cells from cyclophosphamide-treated animals. Our results suggest that a single low dose of cyclophosphamide modulates the expression of galectin-1 and Bcl-2 by tumors, which could in turn influence the apoptotic threshold of spleen mononuclear cells. This mechanism could explain, at least in part, the antimetastatic effect evidenced in our tumor experimental model.

Keywords Galectin-1 · Bcl-2 · Low-dose cyclophosphamide · Lymphoma · Metastasis

G.A. Rabinovich and N. Rubinstein contributed equally to this work.

G.A. Rabinovich · N. Rubinstein
Laboratorio de Inmunogenética,
Hospital de Clínicas "José de San Martín",
Facultad de Medicina, Universidad de Buenos Aires,
Buenos Aires, Argentina

P. Matar · V. Rozados · S. Gervasoni · O.G. Scharovsky (✉)
Instituto de Genética Experimental,
Facultad de Ciencias Médicas,
Universidad Nacional de Rosario,
Santa Fe 3100, (2000) Rosario, Argentina
E-mail: ogs@citynet.net.ar
Tel.: +54-341-4804569
Fax: +54-341-4804569

Introduction

Understanding metastasis, the process by which malignant cells spread from the primary growth site to form secondary tumors in distant organs, is undoubtedly one of the most challenging goals of cancer research because metastasis is the main cause of cancer mortality. The successful completion of each step of the metastatic cascade is determined by unique properties of metastatic cells, as well as host-tumor cell interactions [12].

Both experimental and clinical studies revealed that cyclophosphamide (Cy), an alkylating agent commonly used in cancer chemotherapy [45], exerts an apparently paradoxical effect on host immune response [21]. Large doses of Cy usually bring about an impairment of the host defense mechanisms, along with a reduction of primary tumor mass, therefore leading to severe immunosuppression. However, the administration of low doses of Cy leads to an enhancement of the immune response, both in experimental animals and humans, frequently causing tumor rejection [3, 5]. We have previously shown, in an experimental rat lymphoma model (L-TACB), that a single low dose of Cy has an antimetastatic effect and that this effect can be adoptively transferred with spleen cells of Cy-treated tumor-bearing rats. Moreover, this effect does not take place in immunologically impaired animals [20, 22].

In a search for the molecular mechanisms responsible for these immunomodulatory and antimetastatic effects,

we have recently demonstrated that Cy treatment partially restored the lymphoproliferative response of spleen cells, otherwise reduced during tumor growth, by down-regulating T-cell-derived IL-10 [23, 24] and modulating the Th1/Th2 cytokine balance (Matar et al, submitted).

Galectin-1 (Gal-1), an endogenous β-galactoside-binding protein produced by many types of normal and neoplastic cells [30, 37], plays key roles in several biological and pathological processes, such as cell growth regulation [2, 16,33], cell-matrix interactions [10, 35], and T-cell apoptosis [27, 34, 43,47]. Clear-cut evidence now exists that Gal-1 is implicated in malignant transformation and metastasis of human and experimental tumors [19, 41, 46].

We have demonstrated by using gene therapy strategies that Gal-1 suppressed the inflammatory and autoimmune response by skewing the balance towards a Th2-polarized immune response and ultimately by inducing apoptosis of activated T-cells [36]. This effect has been shown to be mediated mainly by engagement of CD45, CD43, CD7 lymphocyte receptors [27, 28]. The presence of Gal-1 in several lymphoid organs, such as thymus, lymph nodes and spleen, particularly on macrophages, T- and B-cells [6, 32, 34, 49], and at sites of immune privilege and metastasis [19, 41, 46, 48], confirms that this β-galactoside-binding protein participates in the regulation of central and peripheral immune tolerance.

Since Gal-1 has been associated with the invasive and metastatic potential of several tumor cells [14, 19, 46], in the present study we explored the participation of Gal-1 in Cy-induced immunomodulation in a rat experimental lymphoma model. Furthermore, because the response of a cell to a death/proliferation signal is determined by a preset "rheostat" represented by the relative protein levels of members of the Bcl-2 family [1], we also investigated whether metastasis inhibition by Cy is correlated with a modulation of Bcl-2 expression levels. Our results show that a single low dose of Cy induces a modulation of Gal-1 expression by lymphoma cells, with simultaneous up-regulation of spleen Bcl-2, associated with a decrease in spleen cell apoptosis.

Materials and methods

Animals

Adult male and female *IIM e/Fm* rats from the Institute of Experimental Genetics, Faculty of Medical Sciences, National University of Rosario breeding facilities, were used for this study [7]. Animals were fed with commercial food and water ad libitum and were maintained in a 12-h light/dark cycle. All the experiments were developed during the first half of the light cycle. The animals were treated in accordance with the guidelines issued by the Canadian Council on Animal Care.

Drugs and reagents

Cyclophosphamide (Cy) (Endoxan-Asta, Labinca SA, Argentina) was dissolved in sterile distilled water to a concentration of 20 mg/ml and injected at a dose of 10 mg/kg of body weight. Protease inhibitors, DNase-free RNase, Proteinase K, NP-40, 2-mercaptoethanol, molecular weight markers were from Sigma Chemical Co (St. Louis, Mo.). Electrophoretic reagents were from BioRad (Richmond, Calif.). All other chemical reagents were commercially available analytical grade.

Antibodies

Anti-Gal-1 polyclonal Ab was prepared in rabbits as previously described [15]. Anti-Bcl-2 polyclonal Ab was from Santa Cruz Biotechnol (Bcl-2, ΔC21;UK) Horseradish peroxidase-conjugated goat anti-rabbit IgG was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Tumor

Lymphoma TACB (L-TACB) is a poorly differentiated B-cell lymphoma, which arose spontaneously in an inbred *e* rat [13]. It is maintained by serial subcutaneous grafting by trocar of 1 mm^3 L-TACB fragments (approximately 10^6 cells) in syngeneic rats. When L-TACB is s.c. injected, lymph nodes are the exclusive sites of metastatic growth.

Experimental model

The experimental model is illustrated in Fig. 1. Briefly, rats ($n=6$) were implanted s.c. with L-TACB by trocar on day 0. Half of the animals were i.p. inoculated with Cy (10 mg/kg) on day 14 (treated group), and the remaining rats received no further treatment (control group). After 7 days (day 21), treated and control rats were sacrificed and their tumors and spleens were removed. Single-cell suspensions were prepared in PBS by mechanical disruption.

Western blot analysis

To analyze Gal-1 or Bcl-2 expression, tumor or spleen cells were obtained as described above, medium was carefully removed and cells were washed twice with PBS at room temperature. Then, cell pellets were mixed gently with 1 ml ice-cold lysis buffer (PBS containing 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 142.5 mM KCl, 5 mM $MgCl_2$, 10 mM HEPES, pH 7.2) with freshly added protease inhibitor cocktail (0.2 mM PMSF, 0.1% aprotinin, 0.7 μg/ml pepstatin, 1 μg/ml leupeptin)

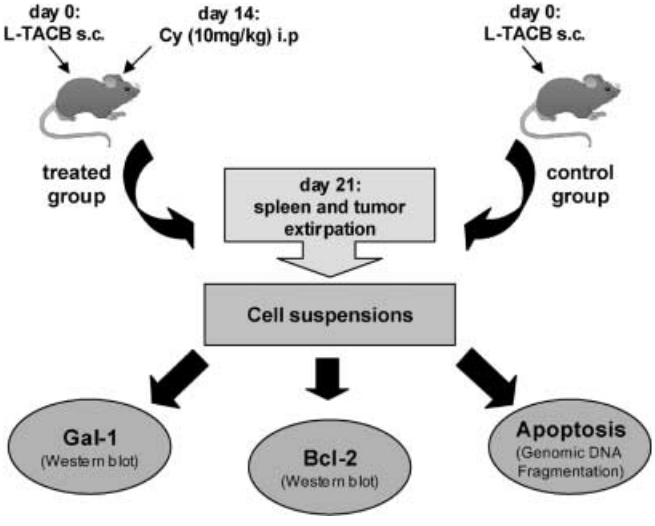


Fig. 1. Experimental model

and incubated for 30 min on ice. Samples were finally centrifuged at 15,000 *g* for 20 min at 4 °C and the supernatant fluid, representing the whole cell protein lysate, stored at –70 °C until use. Protein concentration was estimated by using the micro-BCA Protein Assay reagent kit (Pierce, Rocford, Ill.).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a Miniprotean II electrophoresis apparatus (BioRad, Richmond, Calif.), as described by Laemmli [18]. Briefly, lysates corresponding to tumors or spleens from Cy-treated or non-treated rats (50 μg of protein each) were diluted in sample buffer and resolved on a 15% separating polyacrylamide slab gel. After electrophoresis, the separated proteins were transferred onto nitrocellulose membranes and probed with a 1:1000 dilution of the anti-Gal-1 Ab, as described [10] or a 1:2000 dilution of the anti-Bcl-2 polyclonal antibody (Santa Cruz Biotechnol, UK). Blots were then incubated with a 1:3000 dilution of a horseradish peroxidase-conjugated goat anti-rabbit IgG, developed using enhanced chemoluminiscence detection and finally exposed for 3–5 min to Amersham Hyperfilm (Uppsala, Sweden). Recombinant Gal-1 (rGal-1) was used as a positive control for immunodetection. Control of specific immunoreaction was performed by incubation of the blots with a rabbit preimmune serum without detecting any reactivity. The anti-Gal-1 Ab was monospecific, since it did not recognize other galectins, such as recombinant galectin-3, in Western blot assays. Equal loading and extract degradation were checked by Ponceau S staining and by using an anti-α-tubulin (DM 1A) mAb. Rainbow protein molecular weight markers were from BioRad (Richmond, Calif.). The immunoreactive protein bands were analyzed with a Fotodyne Image Analyzer (Fotodyne, Inc., Hartland, Wis.). Results were expressed as relative densitometric values by means of the Image Quant software.

DNA fragmentation assay

Spleen cells of Cy-treated or non-treated rats were also processed for DNA fragmentation assay, as previously described [34, 36]. In brief, cells were harvested, washed with TNE buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM EDTA, pH 8) and lysed by the addition of 0.5% SDS. Cells lysates were incubated at 56 °C for 3 h in the presence of 100 μg/ml proteinase K. DNA was further purified by successive phenol-chloroform extractions and mixed with 3 M sodium acetate (pH 5.2) and absolute ethanol. The mixture was incubated overnight at –20 °C and the purified DNA was washed, resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and treated with 5 ml of 1 mg/ml DNase free-RNase A for 1 h. Samples were finally resuspended in loading dye and resolved on a 1.5% agarose gel in Tris-Borate-EDTA buffer containing 0.5 μg/ml ethidium bromide.

Statistical analysis

Mann-Whitney test was used to examine the differences between groups.

Results

To explore the cellular and molecular mechanisms implicated in Cy-induced inhibition of lymphoma metastasis, we analyzed expression of Gal-1 by Western blot analysis using a specific polyclonal antibody, on tumors and spleens obtained from Cy-treated or non-treated rats following the experimental model described in Fig. 1. As clearly shown in Fig. 2A, lymphoma cells from three representative non-treated rats expressed high levels of Gal-1 (lanes 1, 2, 3; control group). As has been previously described, the anti-Gal-1 polyclonal Ab recognized not only the 14.5 kDa protein band, but also the homodimeric 29 kDa band. Previously, it has been reported [4, 8, 36, 48] that, even under reducing conditions, scarce amounts of the 29 kDa band persist, since the subunits self-associate by hydrogen bonding. Interestingly, Gal-1 expression was found to be markedly downregulated in tumor cells obtained from Cy-treated L-TACB-bearing rats (Fig. 2A; lanes 4, 5, 6) in comparison with tumor cells derived from non-treated animals (Fig. 2A; lanes 1, 2, 3), as illustrated by the densitometric profile ($P < 0.05$). Recombinant Gal-1 was used as a control of positive immunoreaction (lane 7). Equal protein loading in each lane of the gels was checked by Ponceau S staining and by stripping the filters and re-blotting with DM 1a, an anti-α-tubulin polyclonal Ab (data not shown). This result suggests that Gal-1 expression correlates with the metastatic potential of lymphoma cells and that Cy-induced inhibition of lymphoma metastasis also involves inhibition of Gal-1 expression.

However, treatment with Cy of L-TACB-bearing rats was not able to significantly modify Gal-1 expression by spleen cells (Fig. 2B). Although a trend was observed towards increased Gal-1 expression by spleen cells following Cy treatment, immunoreactivity was heterogeneous, as shown by the densitometric profile in three representative samples (lanes 4, 5, 6).

Since lymphoma tumors express high levels of Bcl-2, which protect cells from apoptosis, and Gal-1 induced T-cell apoptosis through a Bcl-2-, but not Bcl-xL-dependent pathway [38], we also investigated how Cy treatment modulated expression of this anti-apoptotic protein in our experimental model. As shown in Fig. 3A, Bcl-2 expression was downregulated in tumor samples obtained from Cy-treated animals (lanes 4, 5, 6) in comparison to non-treated rats (lanes 1, 2, 3) ($P < 0.05$). This result indicates that Cy treatment may also modulate the apoptotic threshold of lymphoma cells by downregulating Bcl-2 expression levels. In contrast, Bcl-2 was upregulated in spleen cells from Cy-treated animals, as shown by the densitometric profile in Fig. 3B (lanes 4, 5, 6) ($P < 0.05$). This effect was accompanied by a reduction in the intensity of the DNA fragmentation profile of spleen cells from Cy-treated animals (Fig. 3C, treated group vs. control group). This finding supports the idea that Cy treatment could also induce immunomodulation by increasing survival of spleen mononuclear cells.

Discussion

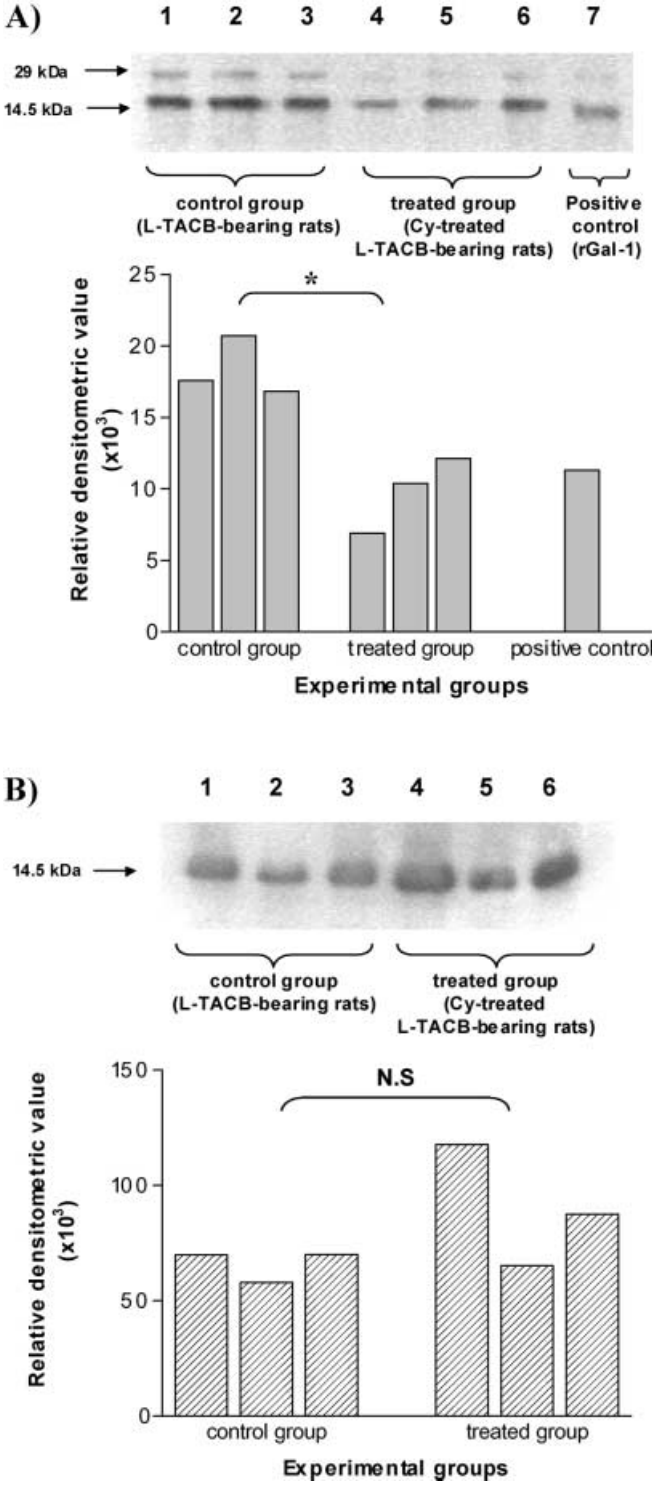
The results presented in this study provide the first experimental evidence regarding Gal-1 and Bcl-2 as molecular targets of the immunomodulatory effects of single low-dose Cy in an experimental rat lymphoma model. While Gal-1 and Bcl-2 were downregulated in tumors after treatment with Cy, an opposite effect was observed in spleen cells following this treatment, where

Gal-1 was not significantly affected and Bcl-2 was up-regulated.

Many chemotherapeutic agents are being successfully used in the treatment of several types of cancer. However, their cytotoxic action affects not only neoplastic cells, but also undesired targets, such as normal rapidly proliferating cells. Thus, the bone marrow is a dose-limiting organ in the administration of high-dose chemotherapy and immunosuppression is the most common side-effect of this kind of treatment [11]. The scarcity in therapeutic resources for preventing or treating metastasis, has stimulated the proposal of several modalities of biological treatment including immunotherapy, which are now under investigation [26]. Several cytotoxic antineoplastic drugs normally used in cancer treatment, such as Cy, can shift the balance from immunosuppression to immunopotentiation, depending on the dose and the administration schedule [21].

We have previously demonstrated in a rat lymphoma model that a single low dose of Cy has an antimetastatic effect [20, 22]. This treatment restored the lymphoproliferative response of spleen cells, otherwise reduced during tumor growth. The levels of Th2 cytokines (i.e. IL-10) and immunosuppressive agents, such as TGF-β and NO produced by spleen cells, decreased after Cy treatment [23]. We found that reduction of IL-10 production after treatment was, in part, responsible for the antimetastatic effect of Cy [24]. Recently, we have demonstrated that low-dose Cy induced a Th2/Th1 shift in the cytokine profile of lymphoma-bearing rats [25].

Galectin-1, an endogenous β-galactoside-binding protein, has recently emerged as a potent immunomodulatory protein [30, 40]. By virtue of its ability to interact with specific glycoconjugates, this protein has been implicated in several biological processes, such as cell proliferation, cell adhesion, apoptosis, metastasis and immunoregulation [2, 36]. Galectin-1 induced apoptosis of activated, mature T-cells and particular subsets of immature thymocytes [28, 29, 34, 43, 47]. Through its ability to inhibit T-cell effector functions, this carbohydrate-binding protein has shown potent anti-inflammatory and immunoregulatory effects in vivo [36, 39, 42]. In this sense, we have recently shown by gene and protein therapy strategies that Gal-1 can suppress the inflammatory and autoimmune response in an



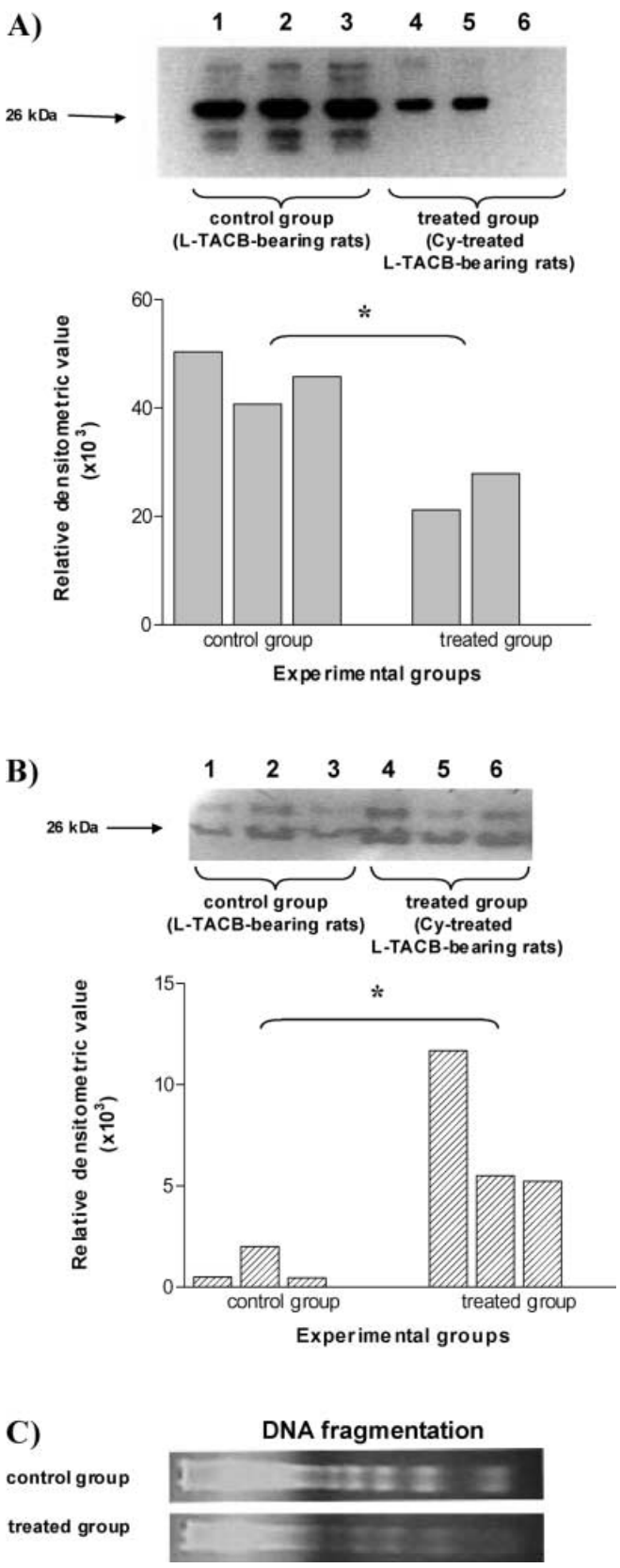
◄

Fig. 2A, B Single low-dose cyclophosphamide modulates Gal-1 expression in lymphoma-bearing rats. Extracts corresponding to tumor cells (**A**) or spleen cells (**B**) from Cy-treated (*lanes 4, 5, 6*) or non-treated rats (*lanes 1, 2, 3*) were prepared and processed for Western blot analysis. Briefly, samples were lysed in the presence of protease inhibitors and equal amounts of protein (50 µg) were subjected to SDS-PAGE on a 15% polyacrylamide slab gel and immunoblotted with a rabbit anti-Gal-1 polyclonal Ab (1:1000). Blots were then incubated with a 1:3000 dilution of a horseradish-peroxidase-conjugated goat anti-rabbit IgG and developed using enhanced chemiluminiscence detection. Recombinant Gal-1 was used as a positive control of immunodetection (*lane 7*). Control of specific immunoreaction was performed by incubation of blots with a rabbit preimmune serum. Immunoreactive protein bands were semi-quantified by densitometry and expressed as relative densitometric values. Equal loading and absence of extract degradation were checked using an anti-α-tubulin (DM 1A) mAb. Results are representative out of three independent experiments with $n=3$ animals per group for each experiment. ($^*P<0.05$)

experimental model of rheumatoid arthritis [36]. Investigation of the molecular mechanisms implicated in this process revealed that Gal-1 treatment could skew the balance towards a Th2-polarized immune reaction and increased T-cell susceptibility to activation-induced cell death. This effect was antigen specific and required signaling via the T-cell receptor (TCR) [31]. Recently, Chung et al [9] showed that Gal-1 acts by antagonizing T-cell responses requiring complete TCR signal transduction, while enhancing those functions requiring only partial TCR-ζ chain phosphorylation, such as apoptosis and cytokine modulation. Previous studies also showed that Gal-1 expression correlated with the acquisition of metastatic phenotype in several types of tumors, such as glioma and prostate carcinoma [19, 41, 46].

In this study we provide experimental data supporting the concept that Cy treatment inhibits Gal-1 expression by lymphoma cells. Since Cy treatment induced a bias towards a Th1 response and Gal-1 is conversely involved in a Th1/Th2 shift [36], one might speculate that Cy would reduce Th2 cytokine production through downregulation of Gal-1. Moreover, it might be also speculated that Gal-1 could be expressed in lymphoma cells as a mechanism of immune escape to induce immunological unresponsiveness by inducing apoptosis of activated T-cells. Supporting this hypothesis, we have found that Bcl-2 upregulation by spleen cells, which indicates decreased apoptotic activity, tightly correlated in each animal with downregulation of lymphoma Gal-1.

Interestingly, while Bcl-2 expression levels were significantly higher in spleen cells from Cy-treated compared to non-treated animals, the opposite effect was demonstrated for tumor cells. Thus, changes in the expression of the antiapoptotic molecule [17], along with other changes yet to be determined, would decrease the apoptotic threshold of tumor cells, but will protect immune cells from death. Therefore, immune cells will be able not only to increase their life-span and survive, but also to switch from a Th2- towards a Th1-cytokine



◄

Fig. 3A–C Single low-dose cyclophosphamide modulates Bcl-2 expression and the apoptotic profile in tumor and spleen cells from lymphoma-bearing rats. Extracts corresponding to tumor cells (**A**) or spleen cells (**B**) from Cy-treated (*lanes 4, 5, 6*) or non-treated rats (*lanes 1, 2, 3*) were prepared and processed for Western blot analysis. Briefly, samples were lysed in the presence of protease inhibitors and equal amounts of protein (50 μg) were subjected to SDS-PAGE on a 15% polyacrylamide slab gel and immunoblotted with an anti-Bcl-2 polyclonal Ab (1:2000). Blots were then incubated with a 1:3000 dilution of a horseradish-peroxidase-conjugated goat anti-rabbit IgG and developed using enhanced chemiluminiscence detection. Immunoreactive protein bands were semiquantified by densitometry and expressed as relative densitometric values. Equal protein loading and absence of extract degradation were checked using an anti-α-tubulin (DM 1A) mAb. Results are representative out of three independent experiments with $n = 3$ animals per group in each experiment. ($^*P < 0.05$). (**C**): DNA fragmentation assay of spleen cells obtained from a representative L-TACB-bearing rat (*control group*) or Cy-treated L-TACB-bearing rat (*treated group*). Following different treatments, samples were harvested, washed and genomic DNA was extracted. Briefly, samples were diluted in loading buffer and resolved on a 1.5% agarose gel. The relative mobility of oligonucleosome-length DNA fragments reflects integer multiples of 180–200 bp

profile, which would proceed the antimetastatic immune response.

The present study allows a further step towards a deeper understanding of the mechanisms of action involved in metastasis treatment with low doses of chemotherapeutic agents [44]. These treatments have the clear advantage of being non-toxic in comparison to high-dose chemotherapy protocols. Further understanding of the cellular and molecular mechanisms implicated in Gal-1 and Bcl-2 modulation by Cy, may have important implications for the therapeutic modulation of immunosuppression and inhibition of metastasis development.

Acknowledgements This study was supported in part by a grant from Fundación Sales for Oncology research. We thank Drs. J. Hirabayashi and K.I. Kasai for providing recombinant Gal-1 and Dr. L. Fainboim for continuous support.

References

1. Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281: 1322
2. Adams L, Kenneth Scott G, Weinberg CS (1996) Biphasic modulation of cell growth by recombinant human galectin-1. Biochem Biophys Acta 1312: 137
3. Awwad M, North RJ (1988) Cyclophosphamide (Cy)-facilitated adoptive immunotherapy of a Cy-resistant tumour. Evidence that Cy permits the expression of adoptive T-cell mediated immunity by removing suppressor T cells rather than by reducing tumour burden. Immunology 65: 87
4. Baum LG, Pang M, Perillo NL, Wu T, Delegaene A, Uittenbogaart CH, Fukuda M, Seilhamer JJ (1995) Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. J Exp Med 181: 877
5. Berd D, Mastrangelo MJ, Engstrom PF, Paul A, Maguire H (1982) Augmentation of the human immune response by cyclophosphamide. Cancer Res 42: 4862
6. Blaser C, Kaufmann M, Muller C, Zimmerman C, Wells V, Mallucci L, Pircher H (1998) β-galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. Eur J Immunol 28: 2311
7. Calderari S, Font MT, Garrocq O, Martinez S, Morini JC, Puche R, Tarrés MC (1991) The inbred IIM/Fm stock. Rat News Lett 25: 28
8. Cho M, Cummings RD (1997) Galectin-1: oligomeric structure and interactions with polylactosamine. Trends Glycosci Glycotechnol 9: 47
9. Chung CD, Patel VP, Moran M, Lewis LA, Miceli MC (2000) Galectin-1 induces partial TCR ζ-chain phosphorylation and antagonizes processive TCR signal transduction. J Immunol 165: 3722
10. Cooper DNW (1997) Galectin-1: secretion and modulation of cell interactions with laminin. Trends Glycosci Glycotechnol 9: 57
11. De Vita VT (1997) Principles of cancer management: chemotherapy: In: De Vita VT, Hellman S, Rosenberg SA (eds) Cancer – Principles & Practice of Oncology, 5th edn. Lippincott-Raven, Philadelphia, p 333
12. Fidler IJ (1997) Molecular biology of cancer: Invasion and metastasis: In: De Vita VT, Hellman S, Rosenberg SA (eds) Cancer – Principles & Practice of Oncology, 5th edn. Lippincott-Raven, Philadelphia, p 135
13. Font MT, Celoria GC, Hinrichsen LI (1983) Genetic analysis of tumor resistance in an inbred line of rats. Medicina (Buenos Aires) 43: 502
14. Gillenwater A, Xu X, El-Naggar AK, Clayman GL, Lotan R (1996) Expression of galectins in head and neck squamous cell carcinoma. Head & Neck 18: 422
15. Hirabayashi J, Ayaki H, Soma G, Kasai KI (1989) Production and purification of a recombinant human 14 kDa β-galactoside-binding protein. FEBS Lett 250: 161
16. Kopitz J, von Reitzenstein C, Burchert M, Cantz M, Gabius HJ (1998) Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. J Biol Chem 273: 11205
17. Kroemer G (1997) The proto-oncogene *bcl-2* and its role in regulation of apoptosis. Nat Med 3: 614
18. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680
19. Lahm H, André S, Hoeflich A, Fischer JR, Sordat B, Kaltner H, Wolf E, Gabius HJ (2001) Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. J Cancer Res Clin Oncol 127: 375
20. Matar P, Celoria G, Font MT, Scharovsky OG (1995) Antimetastatic effect of a single-low dose of Cyclophosphamide on a rat lymphoma. J Exp Clin Cancer Res 14: 59
21. Matar P, Scharovsky OG (1996) Cyclophosphamide bimodal effects in antineoplastic therapy. Rev Bras Cancerol 42: 33
22. Matar P, Rozados VR, Roggero EA, Bonfil RD, Scharovsky OG (1998) Modulation of the antimetastatic response against a rat lymphoma by a single-low dose of cyclophosphamide. Tumor Biol 19: 69
23. Matar P, Rozados VR, González AD, Dlugovitzky DG, Bonfil RD, Scharovsky OG (2000) Mechanism of antimetastatic immunopotentiation by low-dose cyclophosphamide. Eur J Cancer 36: 1060
24. Matar P, Rozados VR, Gervasoni SI, Scharovsky OG (2001) Down regulation of T-cell derived IL-10 production by low-dose cyclophosphamide treatment in tumor-bearing rats restores in vitro normal lymphoproliferative response. Int Immunopharmacol 1: 307
25. Matar P, Rozados VR, Gervasoni SI, Scharovsky OG (2001) Th2/Th1 switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. Cancer Immunol Immunother DOI 10.1007/s00262-001-0237-3
26. Mihich E (2000) Historical overview of biologic response modifiers. Cancer Invest 18: 456
27. Pace KE, Hahn HP, Pang M, Nguyen JT, Baum LG (2000) CD7 delivers a pro-apoptotic signal during galectin-1-induced T cell death. J Immunol 165: 2331
28. Perillo NL, Pace KE, Silhamer JJ, Baum LG (1995) Apoptosis of T cells mediated by galectin-1. Nature 378: 736
29. Perillo NL, Uittenbogaart CH, Nguyen JT, Baum LG (1997) Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. J Exp Med 97: 1851
30. Rabinovich GA (1999) Galectins: an evolutionary conserved family of animal lectins with multifunctional properties. Cell Death Differ 6: 711
31. Rabinovich GA (2000) Apoptosis as a target for gene therapy in rheumatoid arthritis. Mem Inst Oswaldo Cruz 95: 225
32. Rabinovich GA, Castagna LF, Landa CA, Riera CM, Sotomayor CE (1996) Regulated expression of a 16-kDa galectin-like protein in activated rat macrophages. J Leuk Biol 59: 363
33. Rabinovich GA, Modesti NM, Castagna LF, Landa CA, Riera CM, Sotomayor CE (1997) Specific inhibition of lymphocyte proliferation and induction of apoptosis by CLL-I, a β-galactoside-binding lectin. J Biochem 122: 365
34. Rabinovich GA, Iglesias MM, Modesti NM, Castagna LF, Todel CW, Riera CM, Sotomayor CE (1998) Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells; biochemical and functional characterization. J Immunol 160: 4831

35. Rabinovich GA, Ariel A, Hershkoviz R, Hirabayashi J, Kasai KI, Lider O (1999) Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. Immunology 97: 100
36. Rabinovich GA, Daly G, Dreja H, Tailor H, Riera CM, Hirabayashi J, Chernajovsky C (1999) Recombinant Galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. J Exp Med 190: 385
37. Rabinovich GA, Riera CM, Landa CA, Sotomayor CE (1999) Galectins: a key intersection between glycobiology and immunology. Braz J Med Biol Res 32: 383
38. Rabinovich GA, Alonso CR, Sotomayor CE, Durand S, Bocco JL. Riera CM (2000) Molecular mechanisms implicated in galectin-1-induced apoptosis: activation of the AP-1 transcription factor and downregulation of Bcl-2. Cell Death Differ 7: 747
39. Rabinovich GA, Sotomayor CE, Riera CM, Bianco I, Correa SG (2000c) Evidence of a role for galectin-1 in acute inflammation. Eur J Immunol 30: 1331
40. Rabinovich GA, Rubinstein N (2001) Galectins: a novel family of proteins involved in the regulation of the immune response. Implications in immunopathological processes. Medicina (Buenos Aires) 61: 85
41. Raz A, Lotan R (1987) Endogenous galactoside-binding lectins: a new class of functional tumor cell surface molecules related to metastasis. Cancer Metast Rev 6: 433
42. Santucci EL, Fiorucci S, Cammilleri F, Servillo G, Federici B, Morelli A (2000) Galectin-1 exerts immunomodulatory and protective effects on concanavalin-A-induced hepatitis in mice. Hepatology 31: 399
43. Sotomayor CE, Rabinovich GA. (2000) Galectin-1 induces central and peripheral cell death. Implications in T-cell physiopathology. Dev Immunol 7: 117
44. Spreafico F, Vechi A, Colotta F, Mantovani A (1985) Cancer chemotherapeutics as immunomodulators. Springer Semin Immunopathol 8: 361
45. Teicher BA (1997) Antitumor alkylating agents: In: De Vita VT, Hellman S, Rosenberg SA (eds) Cancer – Principles & Practice of Oncology, 5th edn. Lippincott-Raven, Philadelphia, p 405
46. Van Den Brule FA, Waltregny D, Castronovo V (2001) Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. J Pathol 193: 80
47. Vespa GN, Lewis LA, Kozak KR, Moran M, Nguyen JT, Baum LG, Miceli MC (1999) Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. J Immunol 162: 799
48. Wollina U, Schreiber G, Gornig M, Feldrappe S, Burchert M, Gabius HJ (1999) Sertoli cell expression of galectin-1 and -3 and accessible binding sites in normal human testis and Sertoli cell only-syndrome. Histol Histopathol 14: 779
49. Zúñiga E, Rabinovich GA, Iglesias MM, Gruppi A (2001) Regulated expression of galectin-1 during B cell activation and implications for T-cell apoptosis. J Leuk Biol 70: 73