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Immunoregulatory cytokine production by tumor-bearing rat spleen cells and its modulation by bleomycin

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The chemotherapeutic agent bleomycin (BLM) increases cytokine production by mitogen-stimulated healthy rat spleen cells without altering the cellular composition of the spleen. In this study, the chronological production of interleukin (IL)-2, IL-6 and tumor necrosis factor (TNF) by untreated and BLM-treated tumor-bearing rat spleen cells is examined. A significant decrease in the production of both IL-2 and TNF could be observed only 5 days after subcutaneous injection of syngeneic KMT-17 tumor cells. Decrease in cytokine production progressed with time with a slight recovery around day 10 after tumor challenge. Administration of BLM, 5 mg/kg, on day 8, restored IL-2 and IL-6 production and significantly increased TNF production by day 14 of tumor burden as compared with the amounts of cytokine produced by the mitogen-stimulated untreated tumor-bearing rat spleen cells. The response of the tumor-bearing rat spleen cells to concanavalin A (ConA), diminished when compared with that of normal rat spleen cells, could be restored to normal levels by treatment with BLM when examined at low concentrations of mitogen but was unaffected at higher concentrations of ConA. Histological examination of the tumor tissue, following continuous intraperitoneal treatment with BLM, 5 mg/kg, from day 8 to 12, shows disruption of cellular structure with significant infiltration of effector cells as compared with undisrupted organization with no visible infiltration of effector cells in the untreated rat tumors.

Key words: Bleomycin, cytokine production, tumor-bearing rats.

Introduction

Several studies have addressed the importance of the immunological status of the tumor-bearing host in an effort to better understand how neoplastic cells escape host immunological surveillance mechanisms to proliferate and eventually kill the host.[1,2] Important mediators in this field of study are immunoregulatory cytokines which form complicated signalling networks that control the growth, differentiation, recruitment and other cytoregulatory responses of immunological effector cell populations which can inhibit neoplastic cell growth in a variety of ways.[3] Treatment of some human malignant conditions with cytokines such as interferon (IFN)-α in ovarian cancer,[4,5] interleukin (IL)-6 in pleural malignant effusions[6] and tumor-infiltrating lymphocytes transfected with the gene for tumor necrosis factor (TNF)-α against melanoma,[7] have been reported to offer an advantage over more conventional methods of treatment, further illustrating the importance of cytokines as effective mediators of antitumor responses.

Chemotherapeutic agents are intended to inhibit the proliferation of neoplastic cells. However, selective elimination of tumor cells is uncommon and other normal proliferating cells, such as bone marrow cells, may also be affected by the antiproliferative effects of chemotherapy.[8,9] This may result in immunosuppression with a corresponding decrease or imbalance in the production of cytokines and subsequently antitumor effector cell function. Furthermore, some tumors also produce such immunosuppressive factors as prostaglandin E_2 (PGE_2) and transforming growth factor (TGF)-β, and the additive inhibitory effects of chemotherapy may provide a suitable environment for the progressive growth of opportunistic cells within the heterogeneous tumor cell population.[10–12] Through several models developed in our laboratory, we have attempted to modify tumor-induced immunosuppression without eliminating the antineoplastic effects of chemotherapy.

Bleomycin (BLM) is an antitumor antibiotic isolated by Umezawa *et al.* from *Streptomyces verticullus* which, unlike other chemotherapeutic agents, has been reported to have several immunoaugmenting properties.[13] Previous reports have shown that BLM inhibits rat suppressor cell

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function,[14] activates rat tumoricidal macrophages[15] and potentiates rat T cell function.[16] We have also recently reported that BLM, but not two of its newer analogs, significantly increases IL-2, IL-6, IFN and TNF production by mitogen-stimulated healthy rat spleen cells, and that this increase in cytokine production correlated very well with the antitumor effects of the three agents on an antigenic rat fibrosarcoma KMT-17 *in vivo*, even though drug sensitivity assays showed that BLM is significantly weaker than its more potent analogs, peplomycin and liblomycin, *in vitro*.[17,18] In the same study, neither BLM nor its analogs significantly altered rat spleen cell sub-populations in a way that explains the differences in cytokine production. The increase in cytokine production, observed only after treating healthy rats with BLM, is probably a determining factor in inducing effective antitumor responses by effector cells in the KMT-17 tumor-bearing rat, which together with the direct antiproliferative effects of the drug, offer a more effective combined form of treatment. However, whether BLM also increased cytokine production by mitogen-stimulated tumor bearing rat spleen cells has been, up to this point, only speculation.

In this study, cytokine production by mitogen-stimulated tumor-bearing rat spleen cells, examined at several points in time after subcutaneous challenge with syngeneic tumor cells, is described and a number of possible mechanisms for the immunoaugmenting effects observed after treatment with BLM are discussed.

Materials and methods

Animals

Female Wistar King Aptekman (WKA) rats, 6–8 weeks old, were obtained from the Experimental Animal Institute of the Hokkaido University School of Medicine, Sapporo, Japan. Animals were kept under conventional conditions on standard animal feed and water *ad libidum*.

Tumor

The antigenic rat fibrosarcoma, KMT-17, was induced in a WKA rat by 3-methylcholanthrene and maintained *in vivo* by intraperitoneal passage in syngeneic rats every 3 days. The characteristics of this tumor and its variants have been described elsewhere.[19]

Antitumor drug and reagents

BLM was supplied in lyophilized form by Nippon Kayaku (Tokyo, Japan) and dissolved before use in sterile phosphate-buffered saline (PBS). Human recombinant IL-2 was kindly provided by Shionogi (Osaka, Japan) and recombinant TNF was obtained from Suntory Institute for Biomedical Research. Human recombinant IL-6 and its dependent cell line MH-60 were the kind gifts of Professor T. Hirano of Osaka University, Japan. For cell cultures, RPMI 1640 was supplemented with 10% heat-inactivated fetal calf serum (FCS, Filtron, Australia) and 0.05 mM 2-mercaptoethanol and will be referred to as 10% FCS RPMI throughout this report. 3-(4,5-Dimethylthiazol - 2 - yl) - 2,5 - diphenyltetrazolium bromide (MTT, Sigma M-2128) was dissolved in PBS at a dose of 5 mg/ml for use in cell proliferation studies.

Tumor challenge and cytokine production

Rats were subcutaneously injected with 1×10^5 KMT-17 cells and were treated with a single intraperitoneal administration of BLM, 5 mg/kg, 8 days after tumor cell injection as previously reported to induce antitumor effector cell function.[20] Treated or untreated rats were sacrificed 5, 8, 10, 12 and 14 days after challenge with tumor cells and spleens asceptically removed according to the protocol shown in Figure 1. Spleens were teased in loosely fitting ground glass homogenizers and the crude extract passed through several layers of sterile gauze to obtain a single cell suspension. Erythrocytes were lysed by hyposmotic shock with warm Tris buffer, and the remaining spleen cells washed free of buffer and debris with 2% FCS RPMI. The remaining cells were resuspended in 10% FCS RPMI at a density of 1×10^6 cells/ml of medium containing either 1.0 μg/ml concanavalin A (ConA, Sigma C-2631) or 0.1 μg/ml bacterial lipopolysaccharide (LPS). Cells were incubated at 37°C in a 5% CO_2/air humidified atmosphere for 20 h after which culture supernatants were collected and stored at −20°C until thawed for assay of cytokine activity. All determinations performed on spleens taken on days 10 and 14 were carried out at least twice and all

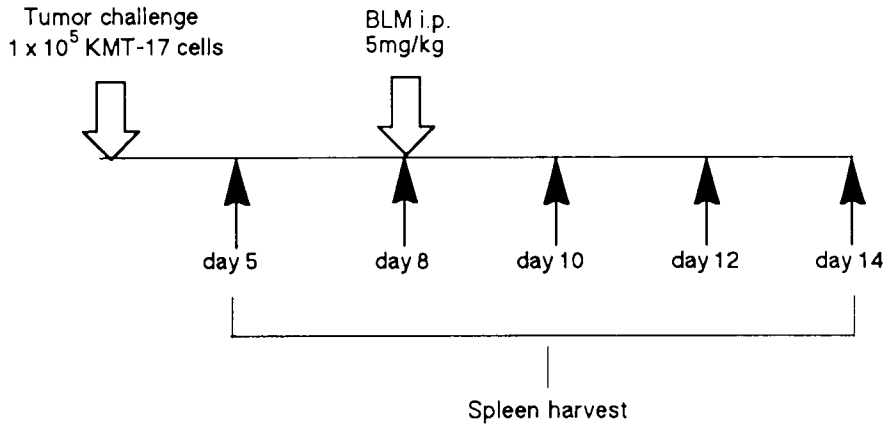

Figure 1. Experimental protocol for the determination of cytokine production by tumor-bearing rat spleen cells. Rats were subcutaneously injected with syngeneic KMT-17 cells and treated with BLM 8 days later. Spleens were taken on the indicated days and erythrocyte-free spleen cells cultured under the stimulatory conditions described in the text.

experiments were performed on triplicate samples of pooled spleen cells obtained from three rats per group.

Assay for IL-2 activity

IL-2 activity present in the ConA-stimulated rat spleen cell culture supernatants was assayed according to the method developed by Gillis[21] with some modification. Briefly, IL-2-dependent CTLL cells, in their logarithmic phase of growth, were washed several times with 2% FCS RPMI and starved of IL-2 by incubation in cytokine-free medium at 37°C for 6 h to enhance the sensitivity of the dependent cells to sample IL-2. Supernatants were serially diluted in duplicate in 100 μl volumes in 96 flat-bottomed well cell culture plates and 4×10^3 CTLL cells were added to each well in equal volumes of medium. Culture plates were incubated at 37°C for 48 h, 10 μl of the MTT solution added to each well and incubation resumed for a further 6 h. Cellular proliferation in response to cytokine was determined according to the method of Mosmann[22] by dissolving the insoluble crystals of formazan, converted from the soluble MTT by proliferating cells, in dimethyl sulfoxide (DMSO). The absorbance of the formazan was measured on a microwell plate reader at 540 nm with a reference wavelength of 600 nm. That amount of IL-2 present in the sample supernatants which supported 50% proliferation of the dependent line was taken to be equivalent to 1 U of IL-2.

Assay for IL-6 activity

IL-6 activity in the samples was determined using a similar method to that employed to determine IL-2 activity. The IL-6-dependent MH-60 cells[23] were washed with large amounts of medium and incubated with serial dilutions of the LPS-stimulated spleen cell culture supernatants in duplicate for 48 h after which MTT was added. The formazan was dissolved in DMSO and absorbance measured at 540 nm as described above. One unit of IL-6 was taken to be that amount of IL-6 which supported 50% proliferation of the dependent line.

Assay for TNF activity

TNF activity in the LPS-stimulated culture supernatants was performed according to the method of Matthews and Neale.[24] Briefly, TNF-sensitive L-929 cells were seeded in 96 flat-bottomed well plates in 100 μl volumes, at a density of 3×10^5 cells per well and incubated at 37°C overnight. Serial dilutions of the LPS-stimulated supernatants were prepared and the L-929 cell supernatants in the microwell plates replaced in duplicate with the diluted samples in the presence of actinomycin D (final concentration 1 mg ml). The plates were incubated for a further 24 h after which supernatants were discarded and the plates stained with crystal violet. Plates were washed, allowed to dry and the dye dissolved in a 33% solution of acetic acid in distilled water. The amount of TNF which exhibited

50% cytotoxicity of the L-929 cells was given a value of 1 U.

In vitro mitogenic response

Erythrocyte-depleted spleen cells were prepared from the spleens of untreated tumor-bearing and BLM-treated tumor-bearing rats obtained 14 days after challenge with tumor cells, and from the spleens of normal rats, and seeded in triplicate in 100 μl volumes in 96 flat-bottomed well plates at 1×10^6 cells/ml of medium containing 0.0, 0.5, 1.0 or 2.5 μg/ml ConA and incubated at 37°C for 72 h in a humidified atmosphere. Cells were pulsed with [^{3}H]thymidine, 1 μCi per well, for the last 18 h of incubation. Cells were then harvested onto glass fiber filter paper and the level of radioactivity determined by scintillation counter.

Histological examination of tumor tissue

Rats were subcutaneously injected with 1×10^5 KMT-17 cells and intraperitoneally treated with BLM, 5 mg/kg, every day for 5 days from day 8 to 12 after tumor challenge. This protocol for treatment of rats bearing this particular tumor has been reported to be the most effective in inducing antitumor effector cell reactions by our group.[20] Tumor tissue was surgically excised from the rats on day 13, embedded in parafin and stained with hematoxylin/eosin for histological examination.

Statistical analysis

All determinations were carried out on triplicate samples obtained from three rats per group and experiments were repeated at least twice unless otherwise stated, with reproducible results. Statistical significance was determined using the Student's *t*-test.

Results

Chronological changes in the production of IL-2

Production of IL-2 by tumor-bearing rat spleen cells stimulated with ConA decreased to 50% that observed in normal rat spleen cell cultures 5 days after challenge with tumor cells, when the tumor was only about 3 × 3 mm in diameter on average. The decrease in IL-2 production progressed with time until it was barely detectible on day 8 with a partial and temporary recovery in cytokine production observed from day 10 of tumor burden. When rats were treated with BLM on day 8, IL-2 production by the spleen cells gradually increased to eventually reach near normal levels by day 14. Figure 2 shows the chronological changes in IL-2 production by rat spleen cells with time after subcutaneous injection of the tumor cells.

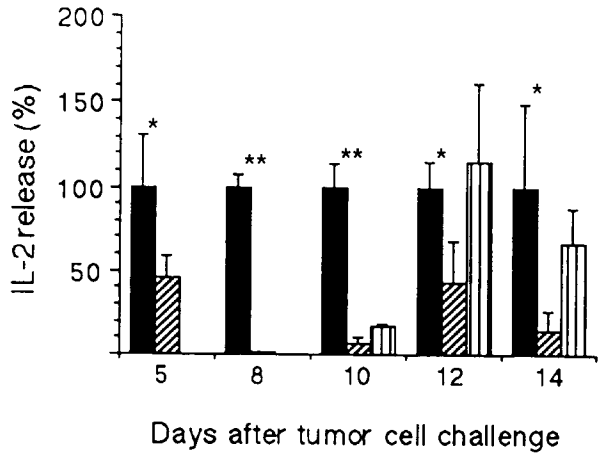

Figure 2. Chronological production of IL-2 by ConA-stimulated tumor-bearing rat spleen cells *in vitro*. Spleen cells were harvested on various days after tumor cell injection and the production of IL-2 after 20–24 h ConA-stimulated culture was determined by bioassay using the IL-2 dependent line CTLL-2. ■, Normal rat; ▨, untreated tumor-bearing rat; ▥, BLM-treated tumor-bearing rat. $^*p < 0.05$ and $^{**}p < 0.001$ versus the IL-2 production in untreated tumor-bearing rat spleen cell cultures.

Chronological changes in the production of IL-6

The production of IL-6 by LPS-stimulated spleen cells was also observed to decrease very early after tumor cell injection when compared with the production of IL-6 by the normal rat spleen cells as shown in Figure 3. IL-6 production gradually decreased with time, again with a slight recovery on day 10, but could be completely restored to levels similar to those observed in the normal controls on day 14 after tumor implantation after the administration of a single intraperitoneal injection of BLM on day 8.

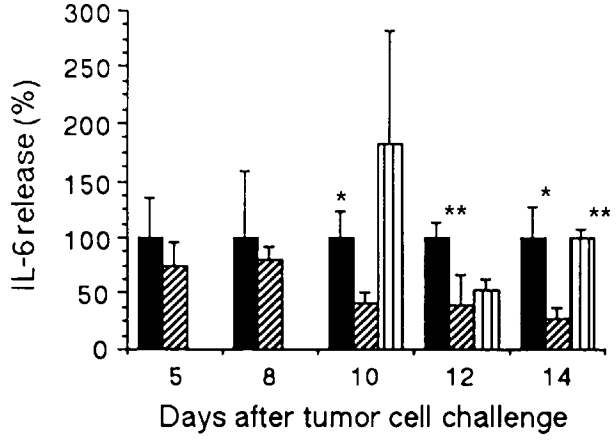

Figure 3. Chronological production of IL-6 by LPS-stimulated tumor-bearing rat spleen cells. Spleen cells were harvested as described in the text and cultured for 20 h in the presence of LPS. The amount of IL-6 released into the medium was determined using the dependent line MH-60. ■, Normal rat; ▨, untreated tumor-bearing rat; ▥, BLM-treated tumor-bearing rat. $^{*}p < 0.02$ and $^{**}p < 0.01$ versus the untreated tumor-bearing rat group.

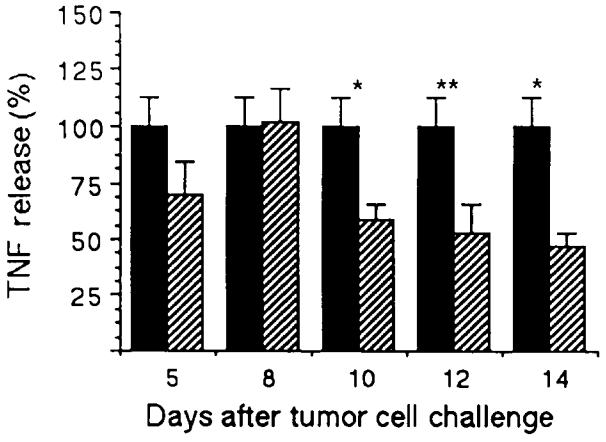

Figure 5. Confirmation of a temporary recovery in the production of TNF by LPS-stimulated spleen cells. Rats were challenged with tumor cells on various days before spleen cell harvest. All spleens were harvested together to eliminate variations in experimental results arising from experimental procedures carried out on different days. Results confirm that a temporary recovery in TNF production occurs between 8 and 10 days after challenge with tumor cells. ■, Normal rat; ▨, tumor-bearing rat. $^{*}p < 0.01$ and $^{**}p < 0.05$ versus the untreated tumor-bearing rat group.

Chronological changes in TNF production

Figure 4 shows the changes in TNF production by the LPS-stimulated rat spleen cells with time following tumor cell injection. TNF production was also observed to decrease by 50% only 5 days after tumor cell challenge with a slight recovery in TNF production on day 10 of tumor burden followed by a progressive decline at least up to day 14, the final time point examined. Treatment with BLM partially restored TNF production on day 14 as compared with the normal controls. In order to confirm whether a recovery in the production of TNF does indeed occur in untreated tumor-bearing rat spleen cell cultures, as can be observed in Figure 4, rats were injected with 1×10^5 KMT-17 cells 5, 8, 10, 12 and 14 days before simultaneous spleen harvest and spleen cells from rats challenged with tumor on different days were examined for their capacity to produce TNF in one experiment. As shown in Figure 5, a recovery in the production of TNF does occur between days 8 and 10 of tumor burden.

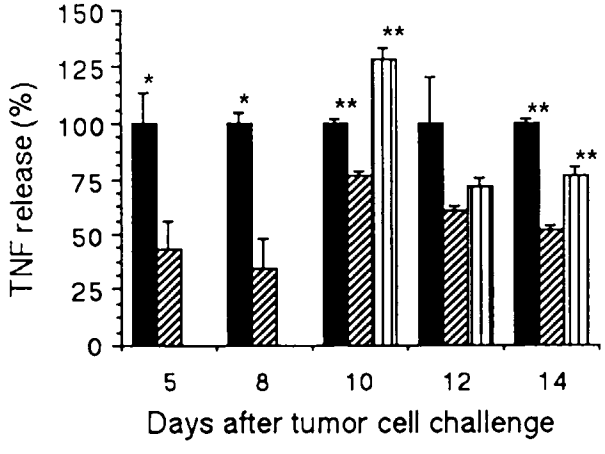

Figure 4. Chronological production of TNF by LPS-stimulated tumor-bearing rat spleen cells. The levels of TNF produced by LPS-stimulated spleen cells obtained from the three groups of rats were determined using the TNF-sensitive line L-929. ■, Normal rat; ▨, untreated tumor-bearing rat; ▥, BLM-treated tumor-bearing rat. $^{*}p < 0.01$ and $^{**}p < 0.05$ versus the untreated tumor-bearing rat group.

In vitro mitogenic response

Figure 6 represents data which shows that spleen cells obtained from untreated tumor-bearing rats, 14 days after injection of tumor cells, exhibit an impaired ability to respond to the mitogenic stimulus of ConA when compared with spleen cells obtained from normal rats. At low concentrations (0.5 μg ml) of ConA, proliferative responses of the tumor-bearing rat spleen cells to mitogen could be restored by administration of BLM. However, at higher concentrations of ConA (1.0 μg ml), the

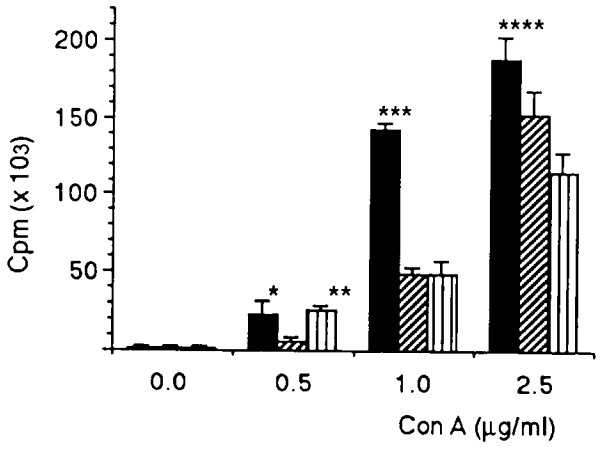

Figure 6. Response of normal, tumor-bearing and BLM-treated tumor-bearing rat spleen cells to the mitogenic stimulus of ConA. Spleens were removed from rats 14 days after injection of tumor cells and stimulated with different concentrations of ConA for 72 h. [^{3}H]Thymidine was added for the final 18 h of culture and the amount of incorporated radioisotope determined by scintillation counter. $^*p < 0.05$ and $^{**}p < 0.01$ versus the untreated tumor-bearing rat group. $^{***}p < 0.01$ versus both treated and untreated tumor-bearing rat groups. $^{****}p < 0.01$ versus the BLM-treated tumor-bearing rat group. □, Normal rat; ▨, untreated tumor-bearing rat; ▥, BLM-treated tumor-bearing rat.

same concentration of mitogen at which cytokine production was examined, BLM-treated and untreated tumor-bearing rat spleen cells responsed similarly to the mitogenic stimulus which was well below the proliferative response of the normal rat spleen cells. At concentrations of 2.5 μg/ml ConA, the proliferative responses of all three groups of spleen cells were similar although the response of the BLM-treated group had a tendency to be lower than that of either the untreated tumor-bearing group or the normal rat group.

Histological examination of tumor tissue

Neoplastic cells within the solid tumor tissue obtained from untreated rats, 13 days after injection of the tumor cells, exhibited irregular size with a number of cells visibly undergoing mitosis. Treatment with BLM for 5 days from day 8 to 12 inclusive considerably disrupted cellular organization when examined on day 13 with no mitotic cells visible. After treatment with BLM, lymphoid cell infiltration was marked while none was observed in the untreated rat tumors. Figure 7 shows a

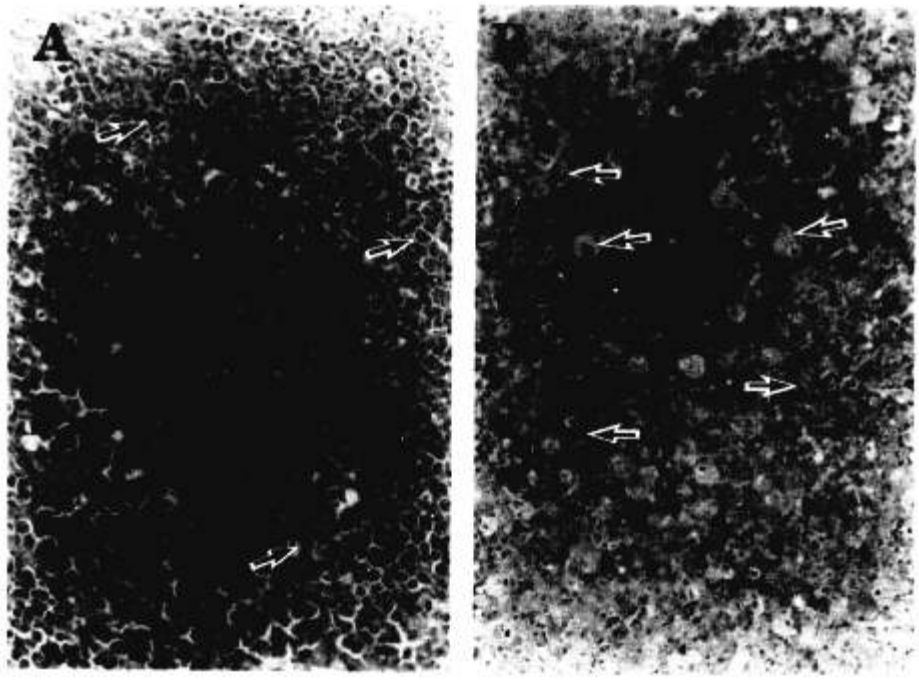

Figure 7. Histology of treated and untreated tumors. Untreated (A) and BLM-treated (B) tumor-bearing rats were sacrificed and their tumors microscopically examined after hematoxylin/eosin staining. M, mitotic cells; L, infiltrating lymphoid cells; F, fibrotic tissue; D, dying cells.

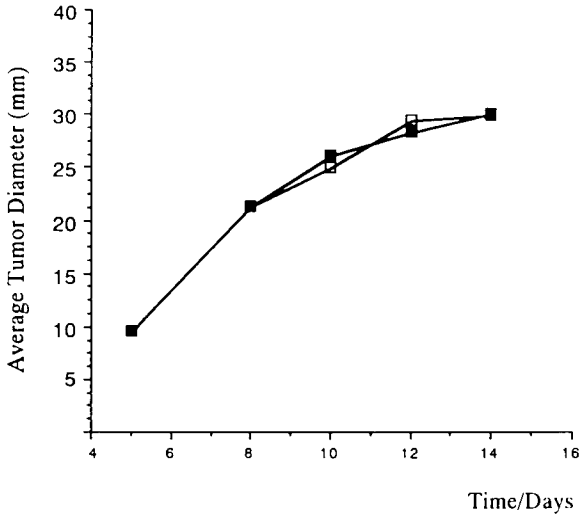

Figure 8. Tumor growth *in vivo*. Tumor growth in untreated and BLM-treated tumor-bearing rats was observed over the same period during which cytokine production was determined. No difference in tumor size could be observed between the treated and untreated groups. ■, untreated tumor-bearing rat; □, BLM-treated tumor-bearing rat.

representative set of histological preparations of both the treated and untreated rat tumors.

In vivo tumor growth

Figure 8 shows tumor growth curves of the treated and untreated groups over the 14 days during which cytokine production was examined. Figure 8 shows that there is no difference in tumor size between the untreated group and the group treated with only one shot of BLM during the period of observation which indicates that the drug-induced increase in cytokine production is not due to a direct effect on the tumor but rather on other immunologically active cells. Histological examination of tumors obtained from the two groups 14 days after tumor implantation did not reveal any significant differences between the two groups (results not shown).

Discussion

In order to develop a system to study tumor-associated immunosuppression and means for its circumvention, we, in our laboratory, have chosen to work with the KMT-17 tumor-bearing rat model and the immunoaugmenting antitumor antibiotic BLM. The *in vitro* production of immunoregulatory cytokines by mitogen-stimulated normal rat spleen cells was found to significantly increase after a single intraperitoneal administration of BLM *in vivo*.[17,18] The *in vivo* antitumor effects of BLM correlated very well with the increase in cytokine production by rat spleen cells observed *in vitro*. However, whether this increase in cytokine production induced by BLM is actually reproducible in the tumor-bearing state, bearing in mind the complex immunological network modulated by the tumor and its products, has been in some doubt. There are several reports describing a variety of immunosuppressed states in tumor-bearing animals,[25-27] and it is the purpose of this study to try to further clarify the immunological events occurring in the tumor-bearing rat model using cytokine production as a parameter, and investigate any possible effect BLM might have on the production of cytokines by tumor-bearing rat spleen cells. Reports from this laboratory have already shown that BLM induces a variety of tumoricidal effector cells in KMT-17 syngeneic tumor-bearing rats[15,28] and it is reasonable to assume that the induction of these antitumor effector cells is propagated by the increased production of stimulatory cytokines as previously reported by Ehrke *et al.*[29] in mice bearing allogeneic tumors.

Although several reports have shown that the administration of exogenous cytokine to tumor-bearing hosts may be advantageous,[30,4,5] and reverses some types of immunosuppression,[31] the protocol used in this present study employs an antitumor antibiotic as the inducer of immunologically-mediated antitumor responses, including production of endogenous cytokine, without compromising the direct antitumor effects of the drug. A recent report from this laboratory shows that γ-radiation-induced immunosuppression greatly diminishes the antineoplastic effects of bleomycin in tumor-bearing rats, further exhibiting the potent immunologically-mediated antitumor effects of the agent in the rat.[17] Histological examination of the tumor shows that BLM causes direct cytotoxic effects on the tumor cells while at the same time inducing lymphoid cell infiltration. A single intraperitoneal administration of BLM does not inhibit tumor cell growth which suggests that the antiproliferative effects of a single administration of BLM on the tumor cells alone cannot explain the increase in cytokine production by the spleen cells and a direct modulating effect on the cells by the drug is more plausible an explanation.

In this study, I show that the potential of mitogen-stimulated tumor-bearing rat spleen cells to produce cytokines is impaired early after subcutaneous injection of the tumor cells. The factor

which suppresses cytokine production by the tumor-bearing rat spleen cells and its source have not been identified in this study. Several groups have reported that tumor cells can inhibit host immunological antitumor responses by producing immunosuppressive factors such as PGE_2 and TGF-β.[12,25] However, the KMT-17 tumor cells used in our system do not produce appreciable amounts of immunosuppressive PGE_2 nor do they produce detectible amounts of TGF-β (results not shown). It remains possible, however, that the tumor produces some other factor which selectively stimulates suppressor cells such as recently described for a tumor-bearing mouse model.[32] This would partially explain earlier observations in this laboratory that show that some of the antitumor effects of BLM in the KMT-17-bearing rat are mediated through the selective elimination of suppressor cells[14] which indirectly implies that suppressor cells are active in the KMT-17 tumor-bearing state. The results of preliminary experiments in this area point an accusing finger at macrophages as being inhibitory to antitumor effector cell function; however, this is the subject of an ongoing investigation and any speculation at this point would be premature.

BLM, whilst increasing cytokine production by normal rat spleen cells, has already been shown by flow cytometry not to have any effect on rat spleen cell subpopulations, eliminating the possibility that BLM stimulates the selective emergence and proliferation of some dominant sub-population which can explain the increase in cytokine production.[17]

Responsiveness of rat spleen cells to the mitogenic stimulus of ConA is diminished in the tumor-bearing state. BLM restores the proliferative responses of the spleen cells to low concentrations of ConA but not to concentrations of mitogen which were used throughout these experiments to determine cytokine production. No differences in proliferative responses to mitogen could be observed between the treated and untreated tumor-bearing rat groups. Spleen cells from both these groups showed a significant impairment in the response to higher concentrations of mitogen when compared with the normal rat spleen cells, with the BLM-treated tumor-bearing rat cells having the least response and, therefore, restoration of cytokine production by BLM in the tumor-bearing state is not the result of a greater response to mitogen by the treated rat spleen cells.

Another possible mechanism for drug-induced increased cytokine production is the enhanced stability of cytokine mRNA through interaction with spleen cell surface molecules such as CD28. A previous report has shown that stimulation of this molecule with activating antibodies increases the stability of cytokine mRNA and subsequently the amount of encoded proteins.[33] That BLM modifies cell surface architecture can be seen from a report which shows that BLM increases cell surface antigen expression *in vivo*.[34]

Schreck *et al.* have shown that NFκB, which is a transcription activator for a number of cytokine encoding genes including that for IL-2, is dissociated from its inactive complex with the inhibitory factor IFκB by active oxyradical species and initiates cytokine production.[35] It is curious to note that BLM also generates active oxygen species[36] which, at the moderate dose applied in this study, might be sufficient to increase cytokine production by both normal and tumor-bearing rat spleen cells without diminishing the antiproliferative effects of the agent on tumor cells.

Although difficulties abound in working with the rat tumor-bearing model, due to the limited number of experimental tools, such as appropriate molecular probes and specific antibodies, researchers in this laboratory are now constructing primers for specific genetic sequences in the rat genome, which will hopefully serve to shed more light on the various immunoaugmenting effects of BLM.

Conclusion

This study describes how cytokine production by rat spleen cells is decreased in the KMT-17 tumor-bearing state but can be restored by the intraperitoneal administration of the immunoaugmenting antitumor antibiotic BLM. Whether this restoration of cytokine production is short- or long-term and whether it is specific for the KMT-17 tumor has not been addressed in this study. However, restoration of cytokine production in the early stages of tumor burden is thought to be an important event in the antitumor effects of BLM before the tumor burden reaches too advanced a stage. The antitumor effects of BLM can be clearly observed from histological examination of the tumor tissue after treatment with the antitumor agent, which shows disruption of the tumor structure with considerable infiltration of lymphocytes whereas lymphocyte infiltration cannot be observed in the untreated rat tumors. It is assumed that these infiltrating cells also produce cytokines

which may recruit and stimulate other antitumor effector cells.

The mechanism for tumor-associated immunosuppression has not been elucidated in this study, but tumor-derived PGE_2 or TGF-β were not found to be involved. The mechanism for KMT-17 induced immunosuppression is now under investigation and, hopefully, some results in this area will be obtained soon.

Acknowledgments

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