Europ. J. Cancer Vol. 15, pp. 1305–1314

0014-2964/79/1101 1305 $02.00/0

Combined Effects of BCG or *Candida albicans* (CA) with Antitumor Agents against a Virus-Induced Lymphoma in Mice*

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Abstract—*Moloney-virus-induced lymphoma LSTRA of BALB/c origin was inoculated i.p. into histocompatible* $CD2F_1$ *mice or into allogenic* $BD2F_1$ *hosts incompatible for multiple minor histocompatibility loci (MMHL). All untreated mice died with comparable median survival times (MST). However, when recipients were subjected to i.p. treatment with BCG or inactivated* Candida albicans *(CA), significant antitumor effects were detected if the non-specific immunoadjuvants (IA) were given before (on* day -14*) and after (on* day $+1$*) tumor challenge.*

The efficiency of IA was found to be higher in MMHL incompatible mice than in $CD2F_1$ *hosts.*

Chemotherapy experiments were conducted in histocompatible mice using IA given according to various treatment schedules, in combinations with 3 nitrosoureas of clinical interest (i.e. BCNU, CCNU and Me-CCNU). Synergistic antitumor effects were evidenced when the antineoplastic agents were associated with IA administered on the "−14 +1" regimen. These results pointed out that the antilymphoma effects of chemotherapy could be amplified by IA only when the treatment schedule included adjuvants administration prior to tumor challenge.

INTRODUCTION

EXTENSIVE experimental and clinical data are presently available suggesting that tumor associated transplantation antigens (TATA) are frequently detectable on the tumor cell membrane [1, 2]. It is conceivable that the host's responses against TATA could play a significant role in the regulation of cancer growth and spread of metastasis. Therefore, attempts to increase immune responses by specific or non-specific stimulation appear to be justified.

A number of studies indicate that BCG is a highly active non-specific immunoadjuvant employed alone [3–6] or in combination with antitumor chemotherapy [7–9]. However, conflicting results have been obtained in several investigations, showing that BCG was inactive in reducing tumor growth [10], or even capable of producing enhancement [11].

It has been proposed that the treatment schedule of the immunoadjuvant with respect to tumor challenge and drug administration could be a critical factor in conditioning the outcome of combination therapy with BCG [12–14]. Therefore, studies have been conducted to analyze in detail the influence of treatment schedules with BCG on tumor growth in mice inoculated with immunogenic lymphoma cells, and subjected to antineoplastic chemotherapy. In addition, parallel experiments were performed with inactivated *C. albicans* (CA) which has been considered to be a good candidate as an immunoadjuvant,

Accepted 26 February 1979.
*This work was supported in part by Progetto Finalizzato Virus, CNR, Rome, Italy, contract number 770025284/1152505 and in part by contract NCI CM 53826 with the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20014, U.S.A.
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since it contains mannan and glucan [15], which were found to enhance host's resistance against experimental tumors [16, 17].

MATERIALS AND METHODS

Mice

Hydrid (BALB/c Cr × DBA/2 Cr)F_1 (CDSF$_1$, $H-2^d/H-2^d$) and (C57B1/6 Cr × DBA/2 Cr)F_1 (BD2F$_1$, $H-2^b/H-2^d$) mice of both sexes, 2–4 months old, were used. The animals were obtained from the Animal Production Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

Tumor

LSTRA, an ascitic lymphoma induced by Moloney leukemia virus in BALB/c mice [18] was carried in BALB/c mice. The tumor was maintained by serial transplantation of neoplastic cells suspended in Medium 199 by i.p. route. Mortality was recorded for at least 60 days after tumor challenge and the presence of ascites and/or generalized lymphoma was confirmed at autopsy.

Drugs

1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(4-trans-methylcyclohexyl)-1-nitrosourea (Me-CCNU), were kindly supplied by Dr. J. B. Wood, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland. BCNU was dissolved in 0.85% NaCl solution immediately before use; CCNU and Me-CCNU were suspended in 0.85% NaCl solution containing 10% Tween 80.

Bacillus Calmette–Guérin (BCG)

Lyophylized Pasteur BCG ($2–6 \times 10^6$ organisms/mg) was furnished by the Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland. The vaccine was dissolved in 0.85% NaCl solution immediately before use and 1 mg injected i.p. in the total volume of 0.2 ml/mouse.

Candida albicans

The cultures were prepared in our laboratory. Vials containing 1×10^9 CA organisms/ml, inactivated with sodium merthiolate (1/10,000), were diluted in 0.85% NaCl solution to obtain 1×10^8 organisms/ml. All injections were given i.p. in a total volume of 0.2 ml/mouse.

Irradiation of tumor cells in vitro

Lymphoma cells (10^8 cells/ml in medium 199) were inactivated *in vitro* by exposure to 8000 rad in sealed glass vials at 4°C, using a ^{60}Co-irradiator (Hot Spot MKIV, Harwell, England) delivering γ-rays at the rate of 1000 rad/min.

Statistical analysis

Differences in survival times were analyzed according to the Mann–Whitney U-test.

RESULTS

Compatible CD2F$_1$ or MMHL-incompatible BD2F$_1$ mice were challenged with 10^5 or 10^6 LSTRA lymphoma cells i.p. (Table 1). Single injections of BCG or CA were administered before and/or after tumor inoculation according to various treatment schedules. Significant growth inhibition was found in either histocompatible or MMHL-incompatible mice when the immunoadjuvants were given on day −14 and +1 (−14+1 schedule) with respect to lymphoma inoculation. On the other hand, when BCG was administered before or after tumor challenge only, no significant increase in survival time was detected.

Experiments were carried out in order to confirm that graft responses can be induced by LSTRA lymphoma in histocompatible CD2F$_1$ [19], or MMHL-incompatible BD2F$_1$ hosts. The animals were sensitized with a single injection of 25×10^7 irradiated (8000 rad *in vitro*) LSTRA cells i.p. Fourteen days later the mice were challenged with 10^5 viable LSTRA cells. The survival times of recipient mice, illustrated in Table 2, show that transplantation resistance was detectable in sensitized CD2F$_1$ and BD2F$_1$ hosts. However, the allogeneic mice were more resistant than histocompatible recipients (group 2 vs 4). This was in line with the hypothesis that BD2F$_1$ mice would recognize virus-coded TATA [19] and additional MMHL alloantigens associated with LSTRA cells.

Further studies were conducted in histocompatible mice challenged with LSTRA lymphoma inoculated with graded doses of BCNU and with BCG, according to various treatment schedules (Table 3). No improvement of the effectiveness of BCNU chemotherapy was found in mice treated with BCG after tumor challenge (groups 13–20, 33–36), or before tumor transplantation and after drug treatment (groups 29–32). However, sig-

Table 1. *Mortality of compatible CD2F1 or MMHL-incompatible BD2F1 mice inoculated with* 10^6 *or* 10^5 *LSTRA lymphoma cells i.p. and subjected to various treatment schedules of BCG or CA*

Number of LSTRA cells*	Treatment i.p. Agent	Schedule	CD2F1 MST§	*P*‖	D/T¶	BD2F1 MST	*P*	D/T
10^6	—	–	8	—	16/16	8	—	16/16
10^6	BCG*	−14	9	C	8/8	9	C	6/8
10^6	BCG	−7	9	C	8/8	9.5	C	5/6
10^6	BCG	+1	8	C	8/8	7.5	C	8/8
10^6	BCG	+9	8	C	8/8	8	C	8/8
10^6	BCG	−7+1	9.5	C	8/8	NT**	—	—
10^6	BCG	−14+1	12	A	8/8	>60	A	3/8
10^6	BCG	+1+9	8	C	8/8	8	C	8/8
10^6	CA‡	−14	8	C	8/8	NT	—	—
10^6	CA	−7	8	C	8/8	8	C	10/12
10^6	CA	+1	8	C	8/8	8	C	7/7
10^6	CA	+9	8	C	8/8	8	C	8/8
10^6	CA	−14+1	9	C	8/8	>60	A	9/21
10^6	CA	+1+9	8	C	8/8	8	C	8/8
10^5	—	—	9	—	8/8	9.5	—	7/7
10^5	BCG	−14+1	15.5	A	8/8	>60	A	0/10
10^5	CA	−14+1	12	A	8/8	NT	—	—

*LSTRA cells were given on day 0.
†BCG given 1 mg/mouse single injection on the day reported in the column, with respect to tumor challenge (day 0).
‡CA cells, 2×10^7/mouse, given single injection on the day reported in the column, with respect to tumor challenge (day 0).
§MST, median survival time.
‖*P*, probability according to Mann–Whitney U-test: A, $P \leqq 0.01$; B, $P \leqq 0.05$; C, $P > 0.05$ (not significant). The statistical analysis was performed comparing mice non treated or treated with BCG or CA.
¶D/T, dead mice at day 60 over total animals tested.
**NT, not tested.

nificant increase of the antineoplastic effectiveness of the nitrosourea was obtained when BCG was given before or before and after tumor challenge, before drug administration (groups 7–8, 11–12, 21–24, 26–28). Again the −14+1 schedule of BCG treatment combined with BCNU chemotherapy appeared to be the most efficient protocol. Data, not reported in Table 3 showed that longer times elapsing between BCG treatment and tumor challenge did not provide any advantage over the −14 +1 scheduling.

Additional studies were conducted with BCNU and two other nitrosoureas of clinical interest, namely CCNU and Me-CCNU. Both BCG or CA were administered adopting the −14+1 treatment schedule which was selected on the basis of the favorable results of the experiments illustrated in Tables 1 and 3. Graded doses of BCNU were given to histo-

Table 2. *Survival of histocompatible* $CD2F_1$ *or MMHL-incompatible* $BD2F_1$ *mice challenged with* 10^5 *cells i.p. of LSTRA lymphoma, presensitized with inactivated (i.e., irradiated with* 8000 *rad* in vitro) *LSTRA cells* 14 days *before tumor transplantation*

Group No.	Host	Presensitization (No. of irradiated LSTRA cells)	MST*	D/T†	P_1‡	P_2
1	$CD2F_1$	no	10	6/6	—	
2	$CD2F_1$	25×10^6	15	5/6	A	—
3	$BD2F_1$	no	10	5/6	C	
4	$BD2F_1$	25×10^6	60	1/6	A	A

*MST, median survival time.
†D/T, dead mice at day 60 over total animals tested.
‡P_1, probability according to Mann–Whitney U-test: A, $P \leqq 0.01$. C, $P > 0.05$ (not significant). The statistical analysis was performed comparing mortality of groups 2–4 with that of group 1.
§P_2, probability calculated as in footnote (‡), comparing mortality of group 2 with that of group 4.

Table 3. Effect of BCG treatment on antitumor efficacy of graded doses of BCNU given i.p. to CD2F1 male mice 5 days after challenge with 10^6 LSTRA lymphoma cells

Group No.	BCG: treatment schedule*	Untreated			Treated with BCNU i.p. on day 5 after challenge 6.5 mg/kg			10.8 mg/kg			18 mg/kg		
		MST†	P‡	D/T§	MST	*P*	D/T	MST	*P*	D/T	MST	P	D/T
1–4	none	8	-	8/8	10	-	6/6	11.5	-	7/7	18	-	8/8
5–8	−14	8	C	8/8	10	C	8/8	14	A	7/7	32	A	6/8
9–12	−7	8	C	8/8	11	C	7/7	14	A	7/7	28	A	7/8
13–16	+1	8	C	8/8	10	C	8/8	11	C	8/8	15.5	C	8/8
17–20	+9	8	C	8/8	10	C	7/7	11.5	C	7/7	14	C	8/8
21–24	−14+1	10	A	8/8	13	B	7/7	16	A	7/7	>60	A	1/8
25–28	−7+1	9	C	8/8	13	B	7/7	15	A	7/7	38	A	5/7
29–32	−7+9	8	C	8/8	10	C	8/8	12	C	7/7	15	C	8/8
33–36	+1+9	8	C	8/8	11	C	8/8	12	C	7/7	16	C	8/8

*BCG given 1 mg/mouse single injection i.p. on the day reported in the column with respect to tumor challenge.

†MST, median survival time.

‡*P*, probability according to Mann–Whitney U-test. A, $P \leqq 0.01$; B, $P \leqq 0.05$; C, $P > 0.05$ (not significant). The statistical analysis was performed comparing mice non treated or treated with BCG.

§D/T, dead mice at day 60 over total animals tested.

compatible $CD2F_1$ mice 5 days after inoculation with 10^6 LSTRA lymphoma cells. Single i.p. injection of BCG or CA was given on day −14+1, or −14 or +1 with respect to tumor challenge.

The results, illustrated in Fig. 1, show that a direct relationship was obtained between the dose of BCNU administration and increase of MST in mice treated with 6.5, 10.8, 18 and 30 mg/kg of the drug. When a dose of

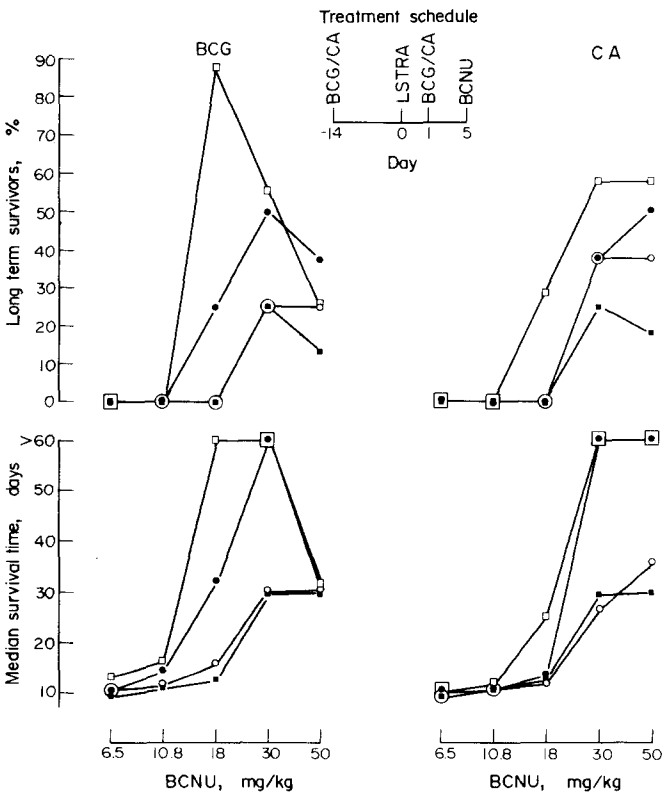

Fig. 1. Combined effects of BCG or CA and BCNU chemotherapy against LSTRA lymphoma in $CD2F_1$ mice. ■—■ *BCNU alone;* ○—○ *immunoadjuvant on* day+1, *BCNU on* day+5; ●—● *immunoadjuvant on* day−14, *BCNU on* day+5; □—□ *immunoadjuvant on* day−14 *and* +1, *BCNU on* day+5.

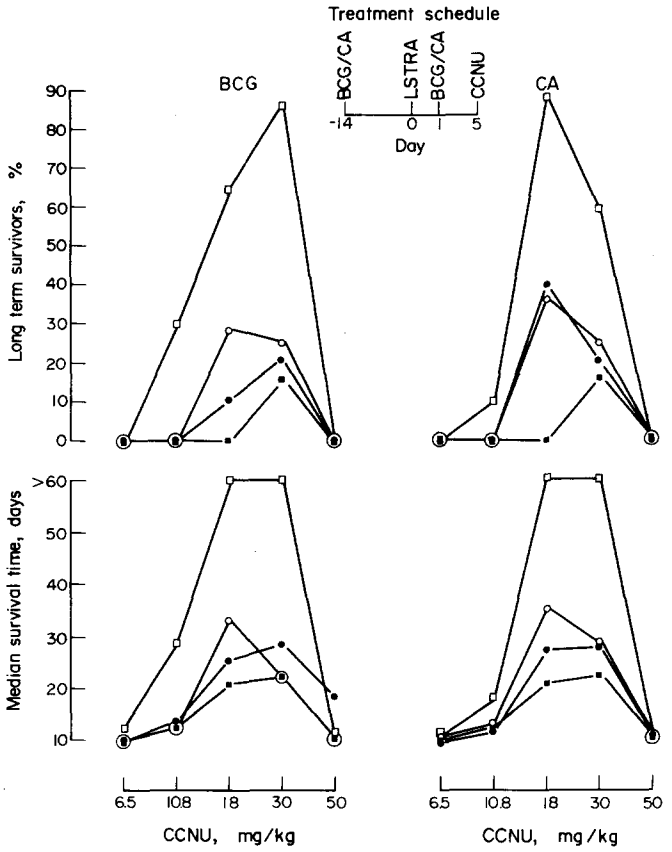

Fig. 2. Combined effects of BCG or CA and CCNU chemotherapy against LSTRA lymphoma in CD2F$_1$ mice. ■—■ *CCNU alone;* ○—○ *immunoadjuvant on* day+1, *CCNU on* day+5; ●—● *immunoadjuvant on* day −14, *CCNU on* day+5; □—□ *immunoadjuvant on* day −14 *and* +1, *CCNU on* day +5.

50 mg/kg of BCNU was used, 11 of 15 mice died without evidence of lymphoma growth, as a result of the toxic effects of this compound [20].

Marked increase of the antitumor efficacy of BCNU was found in mice treated with BCG, given on day−14 or −14+1, but not on day+1 alone. Moreover, significant improvement of the antitumor effectiveness of BCNU (10.8 and 18 mg/kg) was obtained when associated with CA treatment on the −14+1 schedule.

Similar experiments were carried out with CCNU (Fig. 2), or Me-CCNU (Fig. 3). The results confirmed than an optimal schedule for immunoadjuvant administration was the "−14+1" regimen, which provided the best antitumor effects in combination with the optimal dose of 30 mg/kg of both nitrosoureas. Limited or no improvement of chemotherapy was afforded by BCG or CA, when administered on the other treatment schedules (i.e., when given on day−14 or +1 only with respect to tumor challenge).

Further experiments were conducted using BCNU chemotherapy in combination with BCG treatment. A comparative study was performed administering the immunoadjuvant by i.p., s.c. or i.v. routes. The results of a typical experiment, illustrated in Table 4, showed that (a) treatment with BCG alone did not influence substantially the survival times of recipient, histocompatible mice (groups 1–7); (b) marked synergistic effects were found when BCNU treatment was associated with BCG given i.p., especially on the −14+1 schedule (group 10) in accordance with the results of the experiments described previously (Table 3, Fig. 1); (c) the effectiveness of chemotherapy was increased also by BCG given s.c. (groups 11, 12) or i.v. (groups 13, 14). However, the combined treatment was much weaker than that obtainable with BCG given by the i.p. route. In addition, no substantial difference was found between −14 and −14+1 regimen of BCG administration, and no therapeutic advantage over chemotherapy alone was afforded by BCG, when given on day+1 only (data not shown).

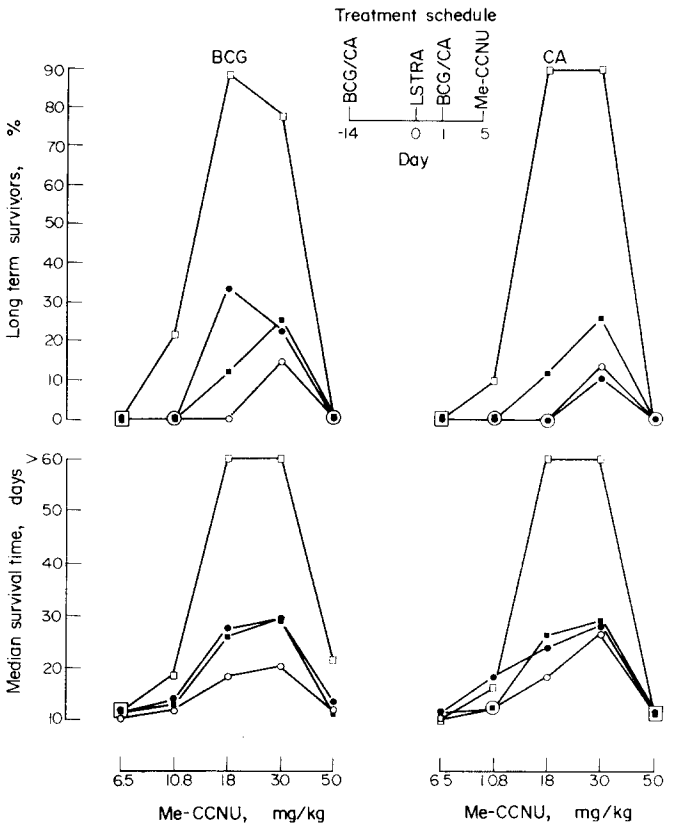

Fig. 3. Combined effects of BCG or CA and Me-CCNU chemotherapy against LSTRA lymphoma in $CD2F_1$ mice. ■—■ *Me-CCNU alone;* ○—○ *immunoadjuvant on* day+1, *Me-CCNU on* day+5; ●—● *immunoadjuvant on* day−14, *Me-CCNU on* day +5; □—□ *immunoadjuvant on* day−14 *and* +1, *Me-CCNU on* day+5.

DISCUSSION

Immunoadjuvants have been studied extensively to determine their therapeutic activity in various host–tumor systems. In particular, the effects of BCG given before (immunoprophylaxis) or after (immunotherapy) tumor challenge have been investigated in a number of experimental neoplasms [3]. It has been shown that immunoprophylaxis is more efficient than immunotherapy in a murine lymphoma [9]. However, immunotherapy with BCG was found to produce marked inhibition of tumor growth in guinea pigs and mice when given along with carcinogen-induced histocompatible neoplastic cells [4, 21]. In addition, BCG given to lymphoma-bearing mice was capable of increasing substantially the effectiveness of antineoplastic agents [7].

In the present study combined effects of chemotherapy and BCG or CA were investigated. The agents were used in prophylactic and/or therapeutic protocols on different treatment schedules. The data illustrated in Table 1 show that the sensitivity of the experimental model used was rather limited. Actually no effect of BCG or CA alone was detected using a challenge of 10^6 LSTRA cells in histocompatible or allogeneic mice. However, when pretreatment was combined with treatment after lymphoma challenge, significant inhibition of tumor growth was obtained. In this case, the treatment schedule was a critical factor, since the timing −14+1 of administration was required for detecting antitumor effects with both BCG or CA.

The therapeutic effectiveness of BCG was slightly superior to that of CA, as evidenced by experiments conducted in mice challenged with 10^6 LSTRA cells i.p. Both agents were used within the range of optimal effective doses. In fact, preliminary dose-effect studies (data not shown) indicated that 1 mg/mouse of BCG and 2×10^7 organisms/mouse of CA were fully effective as immunoadjuvants in histocompatible or allogeneic hosts. No further increase in effectiveness could be obtained using higher or lower doses of the two agents. Tumor growth inhibition was detected in either allogeneic or histocompatible recipients, inoculated with BCG. When recipient mice were subjected to CA treatment, allogeneic

Table 4. Effect of BCG given i.p., s.c. or i.v. on the antitumor efficacy of BCNU in $CD2F_1$ mice challenged intraperitoneally with 10^6 cells i.p. of LSTRA lymphoma

Group No.	BCG Treatment schedule*	Route	BCNU† (mg/kg i.p.)	MST‡	D/T§	P_1‖	P_2¶
1	–	—	—	9	8/8	—	
2	−14	i.p.	—	9	8/8	C	
3	−14+1	i.p.	—	10	8/8	C	
4	−14	s.c.	—	8.5	8/8	C	
5	−14+1	s.c.	—	8	8/8	B	
6	−14	i.v.	—	9	8/8	C	
7	−14+1	i.v.	—	9	8/8	C	
8	—	—	18	17	8/8	A	—
9	−14	i.p.	18	40	6/8	A	A
10	−14+1	i.p.	18	>60	1/8	A	A
11	−14	s.c.	18	22.5	8/8	A	B
12	−14+1	s.c.	18	22	6/8	A	B
13	−14	i.v.	18	25	6/8	A	B
14	−14+1	i.v.	18	24.5	8/8	A	B

*Day of BCG treatment (1 mg/mouse) with respect to tumor challenge (day 0).
†Single injection given on day 5 after challenge.
‡MST, median survival time.
§D/T, dead mice at day 60 over total animals tested.
‖P_1, probability according to Mann–Whitney U-test: A, $P \leqq 0.01$; B, $P \leqq 0.05$; C, $P > 0.05$ (not significant). The statistical analysis was performed comparing the experimental groups with group 1.
¶P_2, probability value, calculated as for footnote (‖) comparing the survival time of mice treated with BCNU alone (group 8), to survival times of BCNU-treated animals subjected to BCG immunotherapy (groups 9–14).

but not histocompatible hosts were protected by immunoadjuvant administration (Table 1). Antitumor effects of CA were detectable in histocompatible $CD2F_1$ hosts when smaller inocula (10^5 cells i.p.) of LSTRA lymphoma were used.

The results obtained with CA appear to be in agreement with experimental data obtained in other animal tumor systems, in which polysaccharides of yeast cell wall were employed [17]. It is well known that CA is rich in mannan and glucan which represent about 18% and 58% respectively of cell wall dry weight [15]. Therefore, it is conceivable that such components could play a significant role in a possible immunoadjuvant activity of CA [16, 17].

The hypothesis that the antitumor effect of BCG and CA in the LSTRA model, could be of immunological nature, seems to be reinforced by the findings of Table 2, showing that the tumor was immunogenic for the host mice used, in accordance with previous reports [19]. Moreover, the observation that lymphoma cells were more immunogenic for allogeneic $BD2F_1$ mice than for histocompatible $CD2F_1$ recipients, seems to be paralleled by the findings that both agents were more efficient in $BD2F_1$ than in $CD2F_1$ mice (Table 1).

The experiments conducted with nitrosoureas and BCG treatment (Table 3) showed that drug efficiency was significantly increased when immunoprophylaxis, but not immunotherapy alone was employed. In this case, the effect of BCG prophylaxis was not detectable in mice not treated with BCNU (Table 1), or inoculated with low dose (i.e., 6.5 mg/kg) of the drug (Table 3). This is consistent with previous observations, showing that additive or synergistic effects between chemotherapy and immunotherapy can be found in tumor bearing animals, when selected doses of antineoplastic agents are used [20]. The chemotherapy experiments (Table 3, Figs. 1–3) provided evidence that immunoprophylaxis plus immunotherapy offered, superior synergistic effects with respect to those detectable with immunoprophylaxis alone, when the agents were combined with chemotherapy, using the 3 nitrosoureas studied. Again, the "−14+1" schedule was the most efficient timing of BCG or CA administration and was capable of increasing significantly the efficacy of BCNU treatment, even at the lowest dose used (6.5 mg/kg, Table 3, Fig. 1). In contrast, when the antineoplastic agent was given in combination with BCG prophylaxis plus BCG therapy, and the latter was administered *after* BCNU treatment (i.e.,

"−7+9" schedule), the efficacy of the combination therapy was drastically reduced, so that it was not superior to that of chemotherapy alone (Table 3). It should be pointed out that these experiments have been performed injecting immunoadjuvants and cancer cells in the peritoneal cavity and, therefore, the effects of BCG and CA may be considered mainly as a result of local rather than generalized activity of these agents.

The experiments performed in order to compare the effectiveness of chemotherapy combined with BCG in relationship to the route of immunoadjuvant administration (Table 4), pointed out that significant improvement of BCNU chemotherapy was afforded by the agent given s.c. or i.v. However, the results showed clearly that the "all i.p." regimen was by far superior to the other schedules used.

Stimulation of T-cell dependent immune responses [22], activation of macrophages [23–25], and increase of natural killer activity [26, 27] have been proposed to account for the antineoplastic activity of BCG. In addition, it was reported that macrophages of BCG-treated mice acquired cytotoxic activity against tumor cells following a second exposure to PPD or BCG *in vitro* [28, 29]. Actually, macrophages can be activated by soluble factors released by T cells subjected to antigenic stimulus [30, 31]. This observation seems to be consistent with the finding that immunoprophylaxis combined with immunotherapy (i.e., rapid and intensive stimulation of T-lymphocytes including memory cells), produces higher antineoplastic effects than immunoprophylaxis or immunotherapy alone. It is conceivable that similar mechanisms could be involved in the therapeutic effectiveness of CA, in accordance with the findings on macrophage activating properties of glucan [32] and mannan [16] and on delayed-type hypersensitivity reactions elicited by CA [33], presumably related to T-cell activation. When BCG or CA were combined with chemotherapy, the net effect may have resulted from complex interactions of the immunoadjuvants and anticancer drugs on host and tumor cells. It has been found that activated cytolytic macrophages or memory T-cells are more radio or chemoresistant than non-prestimulated cells ([34] and Giampietri *et al.*, unpublished data). Moreover, cytotoxic agents can inactivate proliferating precursor cells, rather than activated effector cells [35]. It follows that specific or non-specific immunostimulation should amplify activated macrophage cell population and anti-TATA T. memory cells in hosts bearing immunogenic tumors. In this case, antineoplastic chemotherapy would damage cancer cells more profoundly than the host's cells involved in antitumor immune responses, leading to therapeutic synergism. This seems to be confirmed by the data reported in Table 3, showing that BCG immunoprophylaxis combined with immunotherapy increased drug effectiveness when BCG was administered *before* but not *after* application of the antineoplastic agent. On the other hand, this observation seems to be in contrast with experimental data showing that antitumor agents are markedly immunodepressive when given concomitantly or *after* antigenic stimulation during the proliferative phase of the immune response [36]. However, such depressant activity declines soon [37] and limited impairment of antitumor immunity is expected to occur when enough time elapses between proliferation of immunocompetent clones stimulated by TATA and exposure to cytotoxic drugs [19]. The experimental design adopted in the studies described herewithin, is in line with these concepts, since 5 days elapsed between antigen stimulation (i.e., tumor challenge) and drug treatment.

In conclusion, the studies performed with the animal model described here, concerning the interaction between immunoadjuvants and antitumor agents pointed out that: (a) non-specific immunoadjuvants must be given before tumor challenge for producing synergistic effects with chemotherapy; (b) the timing between immunoadjuvant administration and chemotherapy is critical; (c) the efficacy of antilymphoma combination therapy depends largely on the route of administration of the immunoadjuvant. In addition, the data illustrated in the present report evidence that CA deserves further consideration as an immunoadjuvant, since its antitumor effect is similar to that of BCG, and it does not cause generalized infections, like those produced by viable BCG bacteria, being inactivated organisms.

Acknowledgements—We wish to thank Dr. Joseph G. Mayo and Mr. Clarence Reeder of the Mammalian Genetics and Animal Production Section of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, for breeding and providing the animals. We thank also Dr. J. B. Wood, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland, for providing the necessary drugs. We are grateful also

for the excellent technical assistance of Mr. Mario Andrielli and Ms. B. Kerridge of the Institute of Pharmacology, University of Perugia, 06100 Perugia, Italy.

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