Eur J Cancer Clin Oncol, Vol. 21, No. 9, pp. 1069–1075, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00
© 1985 Pergamon Press Ltd.

Importance of the Concomitant Presence of Palpable MOPC-315 Tumor in Stimulation of Splenocytes by C-type MOPC-315 Virus *in Vitro**

ZIVIA SCHWARZBARD,† RACHEL OPHIR,† TAMAR GOTLIEB-STEMATSKY†‡ and SHLOMO BEN-EFRAIM†§

†*Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv* 69978 *and*
‡*Central Virology Laboratory, Ch. Sheba Medical Center, Tel-Hashomer, Tel-Aviv* 52621, *Israel*

Abstract—*BALB/c mice inoculated with MOPC-315 tumor cells developed an antiviral response against C-type particles extracted from subcutaneous tumors of plasmacytoma-bearing mice as shown by* in vitro *stimulation of spleen cells from tumor-bearing mice by virus-containing preparations. Induction of blastogenic response by virus-containing preparations was found to occur in unfractionated spleen cell populations, the glass-wool non-adherent fraction (depleted of macrophages and tumor cells) and the nylon-wool non-adherent (T-enriched) fraction of spleen cells. The antiviral response was no more detectable in spleens of tumor-bearing mice cured by melphalan. Cured mice developed a strong antitumor immune response as shown by their resistance to challenge with a tumorigenic dose of MOPC-315 tumor cells. However, challenge with tumor cells of cured, resistant mice did not induce reappearance of antiviral response.*

INTRODUCTION

CELL-MEDIATED antiviral response has been shown to occur in a series of experimental systems of animals bearing retrovirus-containing tumors. This applies to murine ecotropic C-type viruses, such as murine leukemia viruses (MuLV) [1] and murine mammary tumor viruses (MuMTV) (for review see [2]), and to chickens bearing tumors induced by Rous sarcoma virus [3–5]. Specificity of the response was ascertained by the finding that cellular immunity to MuMTV but not to MuLV antigens was found in mice bearing virus-induced mammary tumors as measured by blast transformation [6]. The sensitized cells in the blast transformation assay were T cells [7]. It is of interest to mention that animals whose tumors had completely regressed rapidly lost the ability to mount a cellular immune response against virus-containing supernatant fluids of transformed cells [3] and that occurrence of antibodies to the virus may account for this reduction in reactivity [4]. In the case of MuMTV, cellular reactivity was found to decline with the progressive growth of the tumor because of occurrence of adherent suppressor cells [7] and other suppressive factors such as antigens and antibodies, or complexes of the two [8]. In another report [9] it was claimed that glass-adherent spleen cells of tumor-bearing mice may suppress non-specifically mitogen responses of normal T lymphocytes. We also found recently [10] that peripheral blood lymphocytes of patients with multiple myeloma, chronic lymphocytic leukemia, chronic myelocytic leukemia and Burkitt's lymphoma were stimulated by a C-type retrovirus.

The MOPC-315 plasmacytoma originally induced in BALB/c mice was found to release both *in vivo* [11] and *in vitro* [12] virus particles which possess RNA-instructed DNA polymerase and other biochemical and biological features characteristic to C-type retrovirus. Molecular DNA:DNA hybridization experiments with cell-

Accepted 1 March 1985.
*This work was supported by grants awarded by the Moise and Frida Eskanasy Institute for Cancer Research, Tel-Aviv University, the Mayerbaum Fund, Sackler School of Medicine, Tel-Aviv University, and the Kessler Fund of the Israel Cancer Association.
§To whom requests for reprints should be addressed.

ular DNAs of various animal species revealed that MOPC-315 virus genome is related extensively to mouse DNA, pointing to the mouse as its species of origin [13].

Melphalan (L-PAM:L phenylalanine mustard) is a chemotherapeutic drug widely used in clinical oncology, particularly in multiple myeloma [14]. It has been shown [15] that a single injection of a relatively non-immunosuppressive dose of this drug cured most of the mice bearing a large, day 11, subcutaneous MOPC-315 tumor but failed to cure most mice at an early non-palpable tumor stage (day 4 after inoculation). This curative effect of L-PAM was shown to be due to cooperation between the tumoricidal effect of the drug and development of antitumor immunity [15].

This study was undertaken to determine the occurrence of antiviral response against MOPC-315 C-type virus in relation to presence of a local tumor, i.e. in normal mice, in mice inoculated with the tumor and left untreated and in mice inoculated and treated with melphalan. The MOPC-315 tumor was considered by us a suitable model for this study, in view of the facts that it represents a myeloma-like state, the tumor cells contain a C-type virus and the tumor-bearing mice can be cured and rendered resistant to subsequent challenge with tumor cells by chemotherapy.

MATERIALS AND METHODS

Animals

Syngeneic, male BALB/c mice, 8–12 weeks old, were obtained from the breeding colony of the Hebrew University, Jerusalem, Israel.

Tumors

The MOPC-315 plasmacytoma was maintained by serial s.c. inoculations. Routinely, mice were inoculated with 1×10^5 viable tumor cells, a dose which leads to progressively growing tumors that ultimately kill all animals in the group.

Chemotherapy

Melphalan powder (L-PAM) (Burroughs-Wellcome Co., Research Triangle Park, NC, U.S.A.) was used. A concentrated solution of 20 mg/ml was prepared immediately before injection, in acid alcohol (5:1 of 95% ethyl alcohol:2 N HCl). Further dilutions were made in PBS, pH 7.2, and used immediately in order to minimize hydrolysis. A single injection of 7.5 mg/kg L-PAM was given i.p. either 4 days (non-palpable tumor stage) or 11 days after tumor-cell inoculation, when the tumor reached the size of approximately 12 mm diameter. As shown previously [15], a dose of 7.5 mg/kg cured mice bearing a large 11 day tumor but was not effective when injected on day 4 after inoculation. Tumor-bearing mice which received L-PAM on day 11 after inoculation and remained tumor-free 30 days after the tumor inoculation were considered to be cured. Cured mice were challenged with 1×10^6 viable tumor cells s.c. on the contralateral flank 30 days after the first tumor inoculation.

Cell suspensions

Single tumor-cell suspensions were prepared by mechanical disruption of tumor pieces in serum-free RPMI 1640 medium (Grand Island Biological Company, Grand Island, NY, U.S.A.). Single spleen-cell suspensions were prepared by mechanical disruption in Eagle's minimal essential medium (Gibco, Grand Island, NY, U.S.A.). The cells were counted microscopically and the viability determined by exclusion of trypan blue (0.4%).

A fraction containing mostly T and B lymphocytes was separated by passage through a glass-wool column as described [16], except that the medium serum supplement was 10% FCS rather than normal mouse serum. Under these conditions tumor cells and most macrophages were removed because of their adherence to the glass-wool column. A T-enriched population was obtained by passing the glass-wool non-adherent T + B fraction through a nylon-wool column [17]. The non-adherent nylon-wool fraction consisted of more than 98% cells sensitive to the cytotoxic action of monoclonal antibody anti-Thy 1,2 (F7D5 Booth, U.K.) and complement. Inactivation of MOPC-315 tumor cells by mitomycin C (Sigma, St. Louis, MO, U.S.A.) was performed by putting 2×10^7 cells in contact with a final concentration of mitomycin C (75 μg/2×10^7 cells) for 30 min at 37°C as described [18].

C-type virus-containing preparations

Three kinds of preparations obtained from subcutaneous tumors of MOPC-315 were used: crude extracts, purified whole viral particles and disrupted viral particles. The crude extracts were obtained by ultrasonic disintegration as previously detailed [10]. Purified whole viral particles were separated from the crude extracts by sucrose density gradients as described [11]. The purified viral particles were disrupted by treatment with 0.2% Nonidet P-40 (NP-40) for 10 min on ice [10]. The virus content in preparations was determined by a reverse transcriptase test [19]. The protein content of the virus preparations was determined by the method of Lowry *et al.* [20] and expressed in μg protein/ml. The three virus-containing preparations were used in tests for stimulation of spleen-cell cultures. A single batch of each virus

preparation was employed in all experiments throughout the study.

Stimulation tests

Spleen-cell suspensions at a concentration of 1×10^6 viable cells/ml were suspended in RPMI 1640 medium supplemented with 15% fetal calf serum (Gibco, Grand Island, NY, U.S.A.), 0.4 nmol glutamine/l (Fluka, Switzerland), 200 units/ml penicillin and 200 μg/ml streptomycin. Microcultures containing a total of 2×10^5 viable spleen cells/0.2 ml were established in plastic plates with flat-bottomed wells (Nunc, Denmark). The viral preparation at a concentration of 25 μg protein/ml was added at the beginning of the incubation period. Control positive cultures were set by adding PHA (Wellcome, U.K.) at a concentration of 1 μg/culture at the beginning of incubation time and plain cultures (without addition of viral preparation or PHA) served as controls of unstimulated lymphocytes. The cultures were produced in quadruplicate and the plates were incubated for 7 days at 37°C in a humidified atmosphere of 5% CO_2. The degree of stimulation was determined by incorporation of [^{3}H]thymidine (Amersham, U.K.), as previously described [10]. [^{3}H]Thymidine (1 μCi/culture) was added for the last 6 hr of incubation. The cultures were harvested by a semiautomatic microharvester and counted in a Packard beta counter. Mean cpm values of quadruplicates ± S.E. were calculated. The stimulation index (SI) was expressed as the ratio between mean cpm in stimulated cultures and mean cpm in non-stimulated control cultures. SI > 1.9 was regarded as expressing positive lymphocyte blastogenic response.

RESULTS

The degree of stimulation of virus-containing preparations was determined in cultures of spleen cells harvested at various time intervals from mice inoculated with MOPC-315 tumor cells, untreated or treated with melphalan. Normal, non-inoculated mice belonging to the same batch as inoculated animals served as controls.

Spleens derived from normal non-inoculated mice were not stimulated by any of the virus-containing preparations whereas the stimulation index in PHA-treated cultures ranged from 7.9 to 11.1. Spleen cells of tumor-bearing mice taken on day 4 after inoculation (at the non-palpable stage) were not stimulated by the virus-containing preparations whereas spleen cells taken on days 12 and 17 after inoculation (palpable tumors present) responded positively to stimulation by the virus-containing preparations. The response to PHA stimulation was already two-fold lower than normal values on day 4 after inoculation and had decreased gradually by days 12 and 17 after inoculation. The results are presented in Table 1.

It has been shown that spleens of tumor-bearing mice contain large amounts of macrophages and tumor cells at the large MOPC-315 tumor stage [21, 22]. In order to find out whether

Table 1. *Blastogenic response of splenocytes from BALB/c mice inoculated with MOPC-315 plasmacytoma to C-type virus-containing preparations obtained from MOPC-315 tumor cells*

Origin of spleen cells	Blastogenic response in presence of:*	Stimulation index† at various days after inoculation 4	12	17	21	30
Non-inoculated	none	1.0 (2301 ± 384)§	1.0 (1948 ± 581)	1.0 (2790 ± 647)	1.0 (2473 ± 602)	1.0 (2531 ± 689)
	crude MOPC-315 extract	0.6	0.8	1.0	0.9	0.7
	purified virus	1.1	1.4	0.9	0.6	0.8
	disrupted virus	0.6	1.3	1.1	0.6	0.9
	PHA	10.2	11.1	9.3	7.9	8.3
TB‡	none	1.0 (4749 ± 693)	1.0 (7348 ± 1829)	1.0 (6060 ± 891)		
	crude MOPC-315 extract	0.5	2.3	2.3		
	purified virus	0.7	1.9	1.9		
	disrupted virus	0.6	3.2	2.2		
	PHA	5.1	2.9	1.8		

**Crude extract:* ultrasonic disintegrate of MCPC-315 tumor cells; *purified virus:* fraction from crude extract separated by sucrose density gradient; *disrupted virus:* purified viral particles disrupted by Nonidet P-40 treatment. All virus-containing preparations were added at a concentration of 25 μg protein/ml (1×10^6 viable spleen cells); PHA:1 μg/culture of 2×10^5 viable spleen cells.

†Stimulation index: cpm (rate of [^{3}H]thymidine incorporation) in treated cultures/cpm in untreated cultures; cultures were incubated for 7 days; [^{3}H]thymidine (1 μCi/culture) was added for the last 6 hr of incubation; 5–10 parallel samples were run for each possibility.

‡TB: tumor-bearing mice; mice were inoculated s.c. on day '0' with 1×10^5 viable MOPC-315 tumor cells; death of tumor-bearing mice started on day 19 after inoculation.

§( ): mean cpm ± S.E.

*Table 2. Blastogenic response of fractionated populations of splenocytes from BALB/c mice to C-type virus-containing preparations of MOPC-315 tumor cells**

Blastogenic response in presence of:	Stimulation index in populations of normal spleen cells			Stimulation index in populations of TB spleen cells		
	Unfractionated	GWNA‡	NWNA§	Unfractionated	GWNA	NWNA
None	1.0 (3370 ± 251)	1.0 (3451 ± 171)	1.0 (1616 ± 413)	1.0 (7463 ± 496)	1.0 (5206 ± 1304)	1.0 (5954 ± 489)
Crude MOPC-315 extract	0.4	0.5	0.8	2.8	2.8	3.0
Purified virus	0.8	1.0	0.7	2.8	2.9	2.2
Disrupted virus	0.5	0.8	0.9	2.7	3.2	2.5
PHA	10.7	11.7	8.3	2.6	2.8	2.5
MC-MOPC†	0.8	ND‖	0.9	0.5	ND	0.8

*See footnotes to Table 1 for details.
†MC-MOPC: MOPC-315 tumor cells treated with mitomycin C at a concentration of 75 μg/2 × 10^7 cells for 30 min at 37°C; 2 × 10^5 splenocytes were cultured in presence of 2 × 10^5 MC-MOPC cells for 7 days.
‡GWNA: glass-wool non-adherent fraction (mostly T + B cells).
§NWNA: nylon-wool non-adherent fraction (mostly T cells).
‖ND: not done.

*Table 3. Blastogenic response of splenocytes from BALB/c mice to C-type virus-containing preparations obtained from MOPC-315 tumor cells; mice were treated with melphalan after inoculation with MOPC-315 plasmacytoma**

Origin of spleen cells	Blastogenic response in presence of:	Stimulation index at various days after inoculation 12	17	21	30
TB (L-PAM day 4)†	none	1.0 (3490 ± 600)	1.0 (6508 ± 1498)	1.0 (3907 ± 722)	1.0 (4263 ± 801)
	crude MOPC-315 extract	0.5	0.4	1.9	2.0
	purified virus	0.6	0.7	3.5	2.2
	disrupted virus	0.3	0.4	2.5	2.1
	PHA	ND§	1.9	3.4	3.4
TB (L-PAM day 11)‡	none	1.0 (2896 ± 420)	1.0 (7688 ± 1257)	1.0 (4295 ± 1634)	1.0 (3532 ± 631)
	crude MOPC-315 extract	1.7	0.5	0.7	0.8
	purified virus	1.4	0.6	0.7	0.5
	disrupted virus	1.9	0.5	0.9	0.9
	PHA	2.2	1.8	3.1	2.6

*See footnotes to Table 1 for details.
†Tumor-bearing mice were injected with melphalan (L-PAM i.p. 7.5 mg/kg) on day 4 after inoculation (non-palpable stage).
‡Tumor-bearing mice were injected with melphalan (L-PAM) i.p. 7.5 mg/kg on day 11 after inoculation (large-tumor stage).
§ND: not done.

*Table 4. Tumor size of tumor-bearing spleen-cell donors on various days after inoculation**

Group	Size of s.c. tumors (mm) on days: 4	12	17	21	30
TB	NPT†	11.2	23.2		
TB (L-PAM day 4)	NPT	5.5	9.6	16.2	28.7
TB (L-PAM day 11)	NPT	8.7	2.9	NPT	NPT

*See footnotes to Tables 1 and 2 for details; all TB-untreated mice died within 20 days after inoculation.
†NPT: non-palpable tumor.

stimulation induced by virus-containing preparations is not due to the presence of macrophages and tumor cells in spleen-cell suspensions of tumor-bearing mice, stimulation by virus preparations was tested in cultures devoid of macrophages and tumor cells. Macrophages and tumor cells were removed by passage through a glass column. Stimulation by viral preparations was found to occur also in the T + B glass-wool non-adherent fraction as well as in the T-enriched nylon-wool non-adherent fraction. Unfractionated spleen-cell populations as well as the T-enriched nylon-wool non-adherent fraction of spleens from tumor-bearing mice were not stimulated by mitomycin-treated MOPC-315 tumor cells (Table 2).

Mice subjected to melphalan chemotherapy on day 4 after inoculation developed tumors but at a later stage than untreated mice and were still alive with tumors on day 30 after inoculation. The stimulation response to the virus-containing preparation was detectable on days 21 and 30 after stimulation when palpable tumors were already present. At this stage the response to PHA was only approximately half of the normal response (Table 3).

Spleen cells of mice treated with L-PAM on day 11 after inoculation were only slightly stimulated by the virus-containing preparations when taken on day 12 after inoculation and were no more stimulated when taken on days 17, 21 and 30 after inoculation, i.e. at a stage when marked-to-complete regression of tumors was observed (Table 3).

The sizes of local s.c. tumors in spleen-cell donors are given in Table 4.

Tumor-bearing mice, cured by injection of melphalan on day 11 after inoculation, were resistant to challenge with 1×10^6 MOPC-315 tumor cells s.c., performed on day 30 after the first inoculation, but the second injection of tumor cells did not elicit an antiviral response as measured on days 19 and 31 after challenge. On the other hand, an antiviral response was again detected in spleen cells of inoculated, untreated mice. The response to PHA in spleen cells of cured mice remained below levels obtained with spleen cells of normal non-inoculated mice.

The results are presented in Table 5.

*Table 5. Response to stimulation by virus-containing preparations of splenocytes from BALB/c mice cured by melphalan therapy and challenged with MOPC-315 tumor cells**

Origin of spleen cells		Stimulation index (days after challenge)§ 19	31
TB†	none	1.0 (7702 ± 1068)	1.0 (6460 ± 1025)
	crude MOPC-315 extract	1.9	2.5
	purified virus	2.1	3.2
	disrupted virus	2.2	2.6
	PHA	1.5	2.2
TB cured‡	none	1.0 (2943 ± 345)	1.0 (2833 ± 245)
	crude MOPC-315 extract	0.5	0.6
	purified virus	0.6	0.6
	disrupted virus	0.8	0.6
	PHA	4.6	5.0

*See footnotes to Tables 1 and 2 for details.

†Tumor-bearing mice belonged to the same batch as TB-cured mice, i.e. they were approximately 15 weeks old on the day of inoculation; in this group several mice were still alive until day 31 after inoculation with very large tumors (30 mm).

‡Tumor-bearing mice were injected with melphalan, 7.5 mg/kg i.p. on day 11 after the first inoculation; cured mice (without palpable tumors) were challenged with 1×10^6 viable MOPC-315 tumor cells s.c., on day 30 after the first inoculation.

§Days 49 and 61, respectively, after the first inoculation.

DISCUSSION

Cell-mediated antiviral responses were shown to occur in animals bearing virus-containing tumors [1–8] and in certain neoplastic conditions in man [10].

The main finding in the present study is that *in vitro* stimulation of spleen cells by C-type virus-containing preparations derived from MOPC-315 tumor cells was correlated with the concomitant presence of a local subcutaneous MOPC-315 tumor. Thus spleen cells from tumor-bearing untreated mice were stimulated by C-type virus preparations if harvested at a stage when palpable tumors were present, i.e. on days 12 and 17 after

inoculation. The relationship between antiviral response and presence of local tumor was further proven by the finding that, when development of tumor was delayed by injection of melphalan on day 4 after inoculation, the antiviral response was also delayed and was detected only on days 21 and 30 after inoculation, when palpable tumors could be measured. Moreover, the slight antiviral response of spleen cells collected on day 12 after inoculation was not detected in spleens of tumor-bearing mice cured by chemotherapy with melphalan injected on day 11 after inoculation (stage of large palpable tumor). It is of interest to mention that challenge of mice with 1×10^6 viable MOPC-315 cells (containing the C-type virus) did not induce an antiviral response in the cured mice in spite of the fact that these mice exhibited strong antitumor immunity as shown by their resistance to a highly tumorigenic, lethal dose.

We show here that spleen cells of tumor-bearing mice are stimulated *in vitro* by C-type-containing soluble preparations of MOPC-315 tumor cells. In view of the fact that spleens of MOPC-315 tumor-bearing mice contain large amounts of macrophages and tumor cells [21, 22], it might be that the increase in thymidine incorporation in the presence of virus-containing preparations is due to the shift in the composition of the spleen-cell population. In order to investigate this possibility, stimulation by virus-containing preparations was tested in fractions of spleen-cell populations devoid of macrophages and tumor cells. We found that T + B and T-enriched fractions of spleens from tumor-bearing mice were stimulated by viral preparations to the same extent as unfractioned spleen-cell populations (containing macrophages and tumor cells). Therefore it seems likely that the spleen-cell population of tumor-bearing mice stimulated *in vitro* by virus-containing preparations of MOPC-315 tumor cells is essentially a T-cell population. It might also be of interest to mention that whole MOPC-315 tumor cells inactivated by mitomycin did not stimulate spleen-cell populations of tumor-bearing mice. Accordingly, a soluble extract of MOPC-315 tumor cells containing the C-type virus or the MOPC-315 C-type virus alone are responsible for induction of stimulation of spleen cells from tumor-bearing mice.

In view of the findings presented in this paper, it seems plausible that occurrence of antiviral response in cases of tumors induced by virus-containing cells may indicate the presence of an active tumor. Decrease in the degree of antiviral response in a tumor-bearing subject may point out the start of tumor regression after successful chemotherapy or other means. Therefore determination of antiviral response in neoplasia may be of value for indicating the presence of an active tumor and for follow-up of tumor regression.

We previously found [10] that lymphocytes from cancer patients were stimulated *in vitro* by crude extracts of P3HR-1 virus-infected cells but not by purified virus preparations. The present finding showing that purified and disrupted virus preparations are able to stimulate spleen cells of tumor-bearing mice suggest that in the MOPC-315 system the blastogenic response is directed either against the virus itself or against a complex of virus and cell component in which the virus is a major determinant.

The present data suggest that development, after inoculation of tumor cells, of antiviral response is independent of development of antitumor response. It has been reported that immunization with C-type particle preparation can induce protection against MOPC-315 but the possibilities of contaminants with specific tumor antigens in C-type preparations or of acquirement of these antigens by the C-type particles have not been overruled [23]. It is also known [24] that BALB/c mice immunized with purified myeloma protein (tumor-specific transplantation antigen) from mouse plasmacytoma MOPC-315 become resistant to inoculation of the corresponding tumor cells.

We assume that in our experimental conditions the response to C-type virus was elicited by inoculation of tumor cells harboring the virus. However, it is still possible that inoculation with a tumor which does not contain this virus may induce an antiviral response by activating a latent C-type virus of BALB/c mice. This possibility has yet to be investigated.

In conclusion, it seems that, under our conditions, the antiviral response was related to the concomitant presence of a local tumor and was no longer detectable in mice exhibiting a strong antitumor immune response, i.e. resistance to tumor challenge.

Acknowledgement—Dr Schwarzbard wishes to express her gratitude for a fellowship obtained from the Israel Cancer Association.

REFERENCES

1. Lee JC, Ihle JN. Mechanisms of C-type viral leukemogenesis. I. Correlation of *in vitro* lymphocyte blastogenesis to viremia and leukemia. *J Immunol* 1979, **123**, 2351-2358.

2. Moore EH, Long CA, Vaidya AB. Mammary tumor viruses. *Adv Cancer Res* 1979, **29,** 347-418.
3. Israel E, Wainberg MA. Development of cellular anti-tumor immunity in chickens bearing tumors induced by Rous sarcoma virus. *J Immunol* 1977, **118,** 2237-2242.
4. Wainberg MA, Israel E. Immune stimulation of sensitized chicken lymphocytes by avian retrovirus proteins. *J Genet Virol* 1982, **60,** 391-396.
5. Hall MR, Qualtiere LF, Meyers P. Cellular and humoral immune reactivity to tumor-associated antigens in chickens infected with Rous sarcoma virus. *J Immunol* 1979, **123,** 1097-1105.
6. Creemers P, Bentvelzen P. Cellular immunity to the mammary tumour virus in mice bearing primary mammary tumours. *Eur J Cancer* 1977, **13,** 503-510.
7. Creemers P, Bentvelzen P. The role of T and suppressor cells in MTV-directed cellular immunity. *Eur J Cancer* 1977, **13,** 261-267.
8. Creemers P. The role of leukocyte subpopulations in the indirect leukocyte adherence inhibition assay. *Eur J Immunol* 1977, **7,** 48-53.
9. Rudcrynzki AB, Mortensen RF. Suppressor cells in mice with murine mammary tumor virus-induced mammary tumors. I. Inhibition of mitogen-induced lymphocyte stimulation. *JNCI* 1978, **60,** 205-211.
10. Schwarzbard Z, Gotlieb-Stematsky T, Vonsover A, Djaldetti M, Hallak A, Yaniv A. Blastogenic response of lymphocytes derived from patients with hematopoietic malignancies to antigens of a type-C retrovirus isolated from a Burkitt's lymphoma cell line. *Eur J Cancer Clin Oncol* 1983, **19,** 1411-1420.
11. Yaniv A, Kleinman R, Eylan E. RNA-instructed DNA polymerase associated with C-type particles produced *in vivo* by murine myeloma cells. *J Genet Virol* 1976, **32,** 301-309.
12. Yaniv A, Gazit A, Dvir M, Guthmann D, Eylan E. Adaptation of murine MOPC-315 myeloma cells to growth *in vitro* and further characterization of their C-type viruses. *Eur J Cancer* 1978, **14,** 771-779.
13. Yaniv A, Gazit A. Characterization of a murine myeloma (MOPC-315) C-type virus by nucleic acid hybridization. *Virology* 1979, **93,** 256-259.
14. Tribalto M, Arcese W, Colombo R, Franchi A. Chemotherapy in multiple myeloma. Review of recent experience. *Recenti Prog Med* 1981, **71,** 141-145.
15. Ben-Efraim S, Bocian RC, Mokyr MB, Dray S. Increase in the effectiveness of melphalan therapy with progression of MOPC-315 plasmacytomas tumor growth. *Cancer Immunol Immunother* 1983, **15,** 101-107.
16. Lowy I, Bussard AE. A simple method for the removal of antibody forming cells from whole spleen cell population. *J Immunol Methods* 1974, **5,** 107-110.
17. Julius MH, Simpson E, Herzenberg LA. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur J Immunol* 1973, **3,** 645-649.
18. Mokyr MB, Dray S. *In vitro* immunization as a method for generating cytotoxic cells potentially useful in adoptive immunotherapy. In: *Methods in Cancer Research.* New York, Academic Press, 1982, Vol. XIX, 385-417.
19. Schwarzbard Z, Yaniv A, Ianconescu M, Perk K, Zimber A. A reverse transcriptase assay for the diagnosis of lymphoproliferative disease (LPD) of turkeys. *Avian Pathol* 1980, **9,** 481-487.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193,** 265-275.
21. Mokyr BM, Braun DP, Dray S. Augmentation of antitumor cytotoxicity in MOPC-315 tumor bearer spleen cells by depletion of glass-adherent cells prior to *in vitro* activation. *Cancer Res* 1979, **39,** 785-792.
22. Mokyr MB, Hengst JCD, Przepiorka D, Dray S. Augmentation of antitumor cytotoxicity of MOPC-315 tumor bearer spleen cells by depletion of dinitrophenol-adherent cells prior to *in vitro* immunization. *Cancer Res* 1979, **39,** 3928-3934.
23. Kleinman R, Dray S. Protection against MOPC-315 plasmacytoma by immunization with C-type particle preparations. *Cancer Res* 1978, **38,** 223-225.
24. Lynch RG, Graff RJ, Sirisinha S, Simms ES, Eisen HN. Myeloma proteins as tumor-specific antigens. *Proc Natl Acad Sci USA* 1972, **69,** 1540-1544.