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# Immune RNA-mediated Transfer of Tumor Antigen Responsiveness to Unresponsive Peritoneal Exudate Cells from Tumor-bearing Animals[1]

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## SUMMARY

Peritoneal exudate cells (PEC) from mice inoculated 5 to 7 days previously with 1 × 10⁶ MOPC-315 plasmacytoma cells exhibit *in vitro* migration-inhibitory factor reactivity to soluble tumor-associated antigens. By 10 to 14 days of tumor growth, PEC from MOPC-315-bearing mice did not elicit migration-inhibitory factor when stimulated with MOPC-315 tumor-associated antigens but were still capable of migration-inhibitory factor production when stimulated with nontumor antigens. RNA-rich extracts prepared from 5- and 6-day postgrafting tumor bearers were capable of transferring tumor antigen reactivity to both normal PEC and PEC from unresponsive MOPC-315-bearing mice. On the other hand, RNA from unresponsive tumor bearers was incapable of transferring tumor antigen reactivity to normal mouse cells.

## INTRODUCTION

Tumors originating in man and animals elicit tumor-specific, cell-mediated immunity in the host. Still, most neoplasms in the tumor-bearing host persist. One explanation for tumor persistence often advanced is that the cell-mediated antitumor response is insufficient in the primary host to eliminate the existing tumor burden due to poor antigenicity of tumor cells, the adverse effects of regulatory mechanisms on cellular immunity of the host, or immunodepressive effects of tumor cells. Thus, immunotherapeutic approaches for cancer therapy have been designed to potentiate host cell-mediated immunity through specific and nonspecific means. There are several reports in the literature describing the therapeutic effects of specifically sensitized lymphoid cells on existing tumors utilizing cells from syngeneic, tumor-immune donors (6, 15, 23). Human neoplastic disease might be treated immunologically if a source of tumor-immune lymphoid cells can be obtained that are compatible with the host's own immune system. One approach might be to rely on autochthonous cells that have been sensitized to autologous tumor antigens *in vitro*. We have studied the cell-mediated immune response of BALB/c mice to a lethal plasmacytoma tumor (MOPC-315) in an attempt to develop an autochthonous therapeutic model for cancer.

## MATERIALS AND METHODS

**Tumors.** Female BALB/c mice, 8 to 10 weeks old, were obtained from The Jackson Laboratory, Bar Harbor, Maine. Throughout the course of this study, only age-matched female mice were used both for the serial propagation of the tumor and as a source of biological materials for experimentation. The plasmacytoma tumors, MOPC-315, MOPC-300, and MOPC-104E, were obtained from Dr. Paul Heller, Westside V.A. Hospital, Chicago, Ill., and maintained by serial s.c. inoculation in mice. For the purposes of this study, it was determined that the minimum s.c. administered dose of MOPC-315 cells required to kill 100% of tumor-challenged mice was 1 × 10⁴ tumor cells. A dose of 1 × 10⁶ MOPC-315 cells was found to kill all hosts in 16 ± 1.5 days (S.E.); this dose, administered s.c., was used for the experiments presented here.

**Antigens.** The nontumor antigens used in this work included *Mycobacterium tuberculosis* (H37Ra strain; Difco Laboratories, Detroit, Mich.) and a conjugate of DNP-Ig[3] prepared with BALB/c mouse serum by the procedure of Little and Eisen (11). Tumor antigen extracts of plasmacytomas were prepared with 3 M KCl according to the method described by Meltzer *et al.* (14). All antigen preparations were filtered through a 0.22-$\mu$m Millipore filter (Millipore Corp., Bedford, Mass.) and tested for migration-inhibitory effects on nonsensitive peritoneal exudate cells in the MIF assay prior to use in experiments. Soluble TAAg preparations that did not cause inhibition of migration of normal mouse PEC were used at concentrations ranging from 400 to 1000 $\mu$g/ml. Mice immunized with mycobacteria or DNP-Ig were given injections of either 100 $\mu$g of DNP-Ig emulsified in Freund's complete adjuvant or 100 $\mu$g of H37Ra mycobacteria in the hind footpads 2 to 3 weeks prior to testing their PEC for MIF production in the presence of

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[3] The abbreviations used are: DNP-Ig, conjugates of DNP and mouse immunoglobulin; MIF, migration-inhibitory factor; TAAg, tumor-associated antigens; PEC, peritoneal exudate cells; PPD, purified protein derivative; RPMI, Roswell Park Memorial Institute; FCS, fetal calf serum; I-RNA, "immune" RNA; MMI, mean migration index; 5- to 6-day RNA or PEC, RNA or PEC obtained from mice inoculated 5 or 6 days previously with 1 × 10⁶ MOPC-315 tumor cells; 12- to 14-day RNA or PEC, RNA or PEC obtained from mice inoculated 12 or 14 days previously with 1 × 10⁶ MOPC-315 tumor cells.



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PPD or DNP-Ig. Testing with the MIF assay was performed in a single-blind manner.

**Agarose Droplet, Cell Migration Inhibition Assay.** The MIF microassay was essentially that described by Harrington and Stastny (7). PEC were elicited in normal, immunized, or tumor-bearing mice by the i.p. injection of 2 ml mineral oil 3 days prior to sacrifice. The PEC were collected and washed 3 times in Hanks' balanced salts solution. Next, the cells were resuspended in 2 ml of RPMI medium supplemented with 10% FCS (Associated Biomedic Systems, Buffalo, N. Y.) and centrifuged at 1000 rpm in a 3-ml graduated glass centrifuge tube to obtain the packed cell volume. If RNA materials were included in the experiment, the cells were pelleted in serum-free RPMI medium to avoid any RNase activity present in serum and subsequently resuspended in 0.5 ml of the same medium. RNA that had been stored at −20° under ethanol was resuspended in serum-free medium and 200 to 500 µg were added to the PEC in medium. The RNA-cell mixture was then incubated at 37° for 20 min on a shaking water bath. Additional RPMI medium supplemented with 10% FCS was then added to the RNA-treated cells which were then centrifuged at 1000 rpm for 10 min. The medium was removed carefully from above the packed cell pellet of RNA-treated or untreated PEC, and an overlay of double strength medium 199 containing 30% FCS and 0.4% sea plaque agar in a 1:1 ratio of medium to agar was added to the cells so that the final volume-to-volume ratio of cells to medium:agar mixture was 1:4. This solution was drawn into a 50-µl Hamilton microsyringe after which a 2-µl droplet of cells in agar was dispensed into Lab-Tek chamber slides (Lab-Tek Products, Naperville, Ill.) which had been precoated with 1 µl of 0.8% sea plaque agar. The slides were refrigerated at 4° for 8 min and covered immediately with 0.3 ml of RPMI + Tris:EDTA:0.9% NaCl solution which had been supplemented with 100 units/ml penicillin and 50 µg/ml streptomycin. The solutions used to fill the chamber slides contained either medium alone, medium + 50 µg/ml DNP-Ig, 10 µg/ml PPD (Connaught Laboratory, Toronto, Ontario, Canada), or 400 to 1000 µg/ml of the KCl-extracted TAAg preparations. The MOPC-300 and 104E preparations that were used as controls in the experiments had previously been used in our laboratory to immunize mice to the MOPC-300 or 104E tumors. Only preparations that had exhibited the capacity to elicit MIF from tumor-immune mouse PEC (average MMI was 40% for MOPC-300 and 49% for MOPC-104E) were used as a source of unrelated tumor antigens for the present experiments.

**Preparation of RNA Extracts.** RNA was extracted from spleen and lymph nodes of normal or tumor-bearing mice by the method of Scherrer and Darnell (21) with modifications by Thor and Dray (22) and Paque *et al.* (16). At the time of tissue excision, PEC obtained from donor mice were also collected and tested for reactivity to MOPC-315 TAAg in the MIF assay. Only those tissues from animals (5 and 6 days postgrafting) whose PEC were stimulated to produce MIF upon exposure to MOPC-315 TAAg were used as sources of I-RNA. Also, all preparations of RNA were tested prior to use in experiments on normal PEC populations to ensure that the RNA materials were not inhibitory to normal PEC migration. Seven separate RNA preparations from 5- or 6-day tumor bearers, 5 RNA preparations from 12- to 14-day tumor bearers, and 4 RNA preparations from normal mice were used for experiments. One preparation of RNA was prepared from mice grafted with tumor and immunized to mycobacteria 2 days later; this preparation was included in the group of RNA preparations from 12- to 14-day tumor-bearing mice.

**Sucrose Density Gradient Analysis.** RNA preparations used in this work were analyzed by sucrose density gradient centrifugation. The RNA was dissolved in 1.5 to 2.0 ml of 0.11 M sodium acetate buffer (pH 5.1) and the absorbancy at 260 nm was measured. The buffer solution containing 125 to 150 µg of RNA was layered carefully on a 5 to 40% linear sucrose gradient with a Pasteur pipet and the gradients were centrifuged (28,000 rpm) at 4° for 18 hr in a Beckman L2-65 centrifuge with a Spinco SW-41 rotor. The gradients were fractionated on an ISCO Model D density gradient fractionator and analyzed optically on an ISCO Model UA-2 UV analyzer (Instrumentation Specialties Co., Lincoln, Nebr.).

**Analysis of Data.** Lab-Tek slides containing the microassays were placed on a Nikon Model 6-c profile projector, and the areas of migration of PEC were traced at 0 and 48 hr of incubation. The area of migration was calculated as the area of the outer circle of PEC minus the area of the initial cell droplet. The raw values of cell migration for antigen-treated and antigen-untreated cells were utilized to calculate a MMI according to the method of Jureziz *et al.* (8). In a recent statistical study of the macrophage-migration inhibition assay (1), MMI's of 70 to 80% were considered to be inconclusive for migration inhibition, MMI's of 80% or greater were considered to be negative for inhibition of migration, and MMI's of 50% or less were considered to represent positive migration inhibition. The data presented in this report have been analyzed in a manner that is consistent with these recommendations, and all of these experiments have been performed in a single-blind manner to avoid bias in the collection of MIF data.

## RESULTS

**MIF Test for Cell-mediated Immunity against MOPC-315 Plasmacytoma in Mice Grafted with 1 × $10^6$ MOPC-315 Tumor Cells.** Mice were assessed for the capability of developing and retaining specific cell-mediated immunity to the MOPC-315 plasmacytoma. These experiments were performed in 1 of 2 ways. Mice were grafted with tumor cells on Day 0, and at various times after grafting, groups of mice were sacrificed and their PEC tested for MIF production. Conversely, mice that had been inoculated with MOPC-315 on differing days were sacrificed on the same day and tested for MIF production in the presence of various antigens. PEC from mice sacrificed 4 days after grafting were not inhibited in their migration; the average MMI was 105% when incubated with soluble MOPC-315 TAAg (Table 1). On the other hand, positive inhibition of migration was observed with PEC collected from mice at 5, 6, or 7 days after tumor grafting; average MMI's were 58, 31, and 32%, respectively (Table 1). PEC were not inhibited in their migration when incubated with tumor antigens

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Table 1

*MIF test for tumor-specific cellular immunity to MOPC-315 TAAg with PEC from MOPC-315-bearing mice*

Mice were given injections of 1 × 10⁶ MOPC-315 cells 4 to 14 days prior to assay. All experiments were performed with pooled PEC from 8 to 10 mice.

| | | MMI of PEC tested with | | | | | |
|---|---|---|---|---|---|---|---|
| | | MOPC-315 TAAg[a] | | MOPC-300 TAAg[b] | | DNP-Ig[c] | |
| Days after grafting | No. of experiments | Mean MMI (%) | range (%) | Mean MMI (%) | Range (%) | Mean MMI (%) | Range (%) |
| 0 | 10 | 110 ± 5[d] | 95–140 | | | | |
| 4 | 4 | 105 ± 5 | 90–124 | | | | |
| 5 | 5 | 58 ± 3 | 48–65 | | | 89 ± 4 | 80–97 |
| 6 | 8 | 31 ± 2 | 29–40 | 118 ± 6 | 90–136 | 90 ± 5 | 85–102 |
| 7 | 6 | 32 ± 3 | 29–35 | 110 ± 5 | 105–120 | 95 ± 2 | 90–106 |
| 9 | 3 | 70 ± 2 | 64–75 | | | 94 ± 3 | 78–90 |
| 10 | 5 | 96 ± 4 | 87–106 | 105 ± 4 | 95–115 | | |
| 12 | 4 | 101 ± 3 | 98–102 | 107 ± 4 | 100–115 | | |
| 14 | 6 | 99 ± 6 | 80–118 | 111 ± 8 | 86–140 | 85 ± 3 | 79–96 |

[a] KCl-extracted MOPC-315 TAAg, 400 to 1000 μg/ml.
[b] KCl-extracted MOPC-300 TAAg, 400 to 1000 μg/ml.
[c] DNP-Ig, 50 μg/ml.
[d] Mean ± S.E.

capable of stimulating MIF production from MOPC-300 immune cells during the 4- to 7-day interval (Table 1). At Day 9, the inhibition of migration (70%) was inconclusive for MIF production, and by day 10, inhibition of migration from PEC of tumor-bearing mice could not be detected; average MMI's were 96% (Table 1). By Day 14, the loss of tumor-specific cellular immunity to MOPC-315 TAAg was clearly demonstrated; average MMI's were 99% (Table 1). Similarly, inhibition of migration of PEC could not be detected in PEC from MOPC-315 bearers that were stimulated with MOPC-300 TAAg preparations or DNP-Ig (Table 1). The pattern of the development and subsequent loss of cell-mediated immunity to MOPC-315 TAAg in tumor-bearing mice is shown in Chart 1.

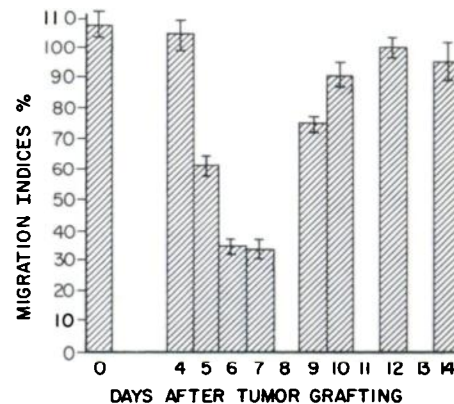


Chart 1. The pattern of the development and subsequent loss of tumor-specific cell-mediated immunity to MOPC-315 TAAg in BALB/c mice grafted with 1 × 10⁶ MOPC-315 plasmacytoma. *Bars*, S.E.

**Specificity of Loss of Cell-mediated Immunity to MOPC-315 TAAg.** To test whether the loss of cellular immunity to MOPC-315 TAAg that is seen by 10 days of tumor growth is specific for tumor antigens, we inoculated mice with 1 × 10⁶ MOPC-315 cells on Day 0 and immunized them with either mycobacteria or DNP-Ig on day 2. Fourteen days after tumor grafting, these animals were sacrificed and their PEC were tested for cellular responsiveness to MOPC-315 TAAg, PPD, or DNP-Ig in the MIF assay. It was determined that PEC from mice immunized with mycobacteria or DNP-Ig were significantly inhibited in their migration upon testing with PPD or DNP-Ig (average MMI was 43% for PPD and 38% for DNP-Ig). When PEC from mice grafted with tumor and immunized to non-tumor antigens were incubated with soluble MOPC-315 TAAg, inhibition of migration could not be detected; average MMI's were 95% (Table 2). On the other hand, when these PEC were incubated with the antigen used for immunization, inhibition of migration was demonstrated; average MMI's were 35% for DNP-Ig and 38% for PPD (Table 2).

**Conversion of Nonsensitive PEC with RNA from Mice Grafted with 1 × 10⁶ MOPC-315 Cells 5 or 6 Days Previously (5- to 6-Day RNA).** PEC were collected from 10 BALB/c mice, washed in Hanks' balanced salts solution, and divided into 3 tubes. One tube received 200 to 500 μg of normal RNA, 1 tube received 200 to 500 μg of 5- to 6-day I-RNA, and 1 tube did not receive RNA. After incubation, the cells were tested for antigen responsiveness in the MIF assay. For insurance that both the RNA preparations and the KCl-extracted TAAg preparations were not inhibitory to macrophage migration, 1 group of microdroplet cultures consisted of RNA-treated cells in medium and 1 group of cultures consisted of untreated cells incubated with soluble tumor antigen. When nonsensitive PEC were treated with 5- to 6-day I-RNA and incubated in medium alone, the cells migrated normally; the average MMI was 94% (Table 3). Normal mouse cells stimulated with soluble MOPC-315 TAAg also migrated comparably to the control cultures; the average MMI was 89% (Table 3). In 2 experiments in which nonsensitive PEC were treated with 5- to 6-day I-RNA and stimulated with MOPC-104E antigen, the cells migrated normally; the average MMI was 112% (Table 3). Also, when normal cells were incubated with normal RNA and stimulated with MOPC-315 TAAg, the cells were not inhibited in



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their migration; the average MMI was 87% (Table 3). On the other hand, when nonsensitive cells were treated with 5- to 6-day I-RNA and stimulated with MOPC-315 TAAg, the cells were clearly inhibited in their migration; the average MMI was 40% (Table 3). In some experiments, 12- to 14-day RNA was also tested with the same cells; these results are included in the data presented in Table 5.

**Conversion of Unreactive PEC from MOPC-315-bearing Mice to Tumor Antigen Responsiveness with 5- to 6-Day I-RNA.** Since it was found that mice grafted with 1 × $10^6$ MOPC-315 cells lose the capacity to react *in vitro* to soluble TAAg by 10 to 14 days of tumor growth, it was of interest to determine whether these unreactive cells could be converted to tumor reactivity by 5- to 6-day I-RNA. PEC from mice grafted 14 days previously with MOPC-315 cells were incubated with 5- to 6-day I-RNA and tested for MIF production in the presence of various antigens. For insurance that the cells utilized in these experiments had truly lost the capacity to be stimulated with soluble tumor antigen, 1 group of microdroplet cultures consisted of untreated cells incubated with MOPC-315 TAAg. These cells migrated comparably to the controls; the average MMI was 104% (Table 4). Similarly, when unreactive cells were treated with 5- to 6-day I-RNA and incubated in medium alone, the cells migrated normally; the average MMI was 97% (Table 4). In 3 experiments consisting of unreactive PEC treated with normal RNA and stimulated with MOPC-315 TAAg, inhibition of migration was not detected; the average MMI was 95% (Table 4). However, when unreactive PEC from terminal MOPC-315 bearers were treated with 5- to 6-day I-RNA and stimulated with MOPC-315 TAAg, the cells were clearly inhibited in their migration; the average MMI was 45% (Table 4). Thus, it appears that 5- to 6-day I-RNA is capable of converting unresponsive PEC from terminal MOPC-315 bearers to tumor antigen responsiveness.

**Failure to Convert Normal Mouse PEC to Tumor Antigen Responsiveness with RNA from Mice Grafted with 1 × $10^6$ MOPC-315 Cells 12 to 14 days Previously.** To investigate the possibility that unresponsive terminal tumor-bearing mice still contain I-RNA for MOPC-315 immunity, RNA was prepared from mice grafted 12 to 14 days previously with 1 × $10^6$ MOPC-315 cells (12- to 14-day RNA). When nonsensitive mouse PEC were treated with RNA of terminal tumor bearers and incubated in medium alone, the cells migrated normally; the average MMI was 99% (Table 5).

PEC treated with 12- to 14-day RNA and stimulated with MOPC-315 TAAg were also found to migrate comparably to the controls; the average MMI was 98% (Table 5). Similarly, when cells were treated with normal RNA and stimulated with MOPC-315 TAAg, or when untreated cells were stimulated with MOPC-315 antigens, the cells did not demonstrate inhibition of migration; average MMI's were 91% in both cases (Table 5). An added control was performed in these experiments to ensure that the 12- to 14-day RNA preparations were biologically active. In these experiments,

Table 2

*MIF test for cellular immunity to MOPC-315 TAAg, PPD, or DNP-Ig with PEC obtained from MOPC-315-bearing, immunized BALB/c mice*

MOPC-315 TAAg, 400 to 1000 μg/ml; PPD, 10 μg/ml; and DNP-Ig 50 μg/ml. All experiments were performed with pooled PEC from 5 mice, and the PEC were collected and assayed on Day 14 posttumor grafting. Mice were given injections on Day 0 with 1 × $10^6$ MOPC-315 and on Day 2 with mycobacteria or DNP-Ig in CFA as described in text.

| Experiment | MMI of PEC (%) tested with | | |
|---|---|---|---|
| | MOPC-315 TAAg | DNP-Ig | PPD |
| 1 | 102 ± 4[a] | 33 ± 3 | |
| 2 | 87 ± 4 | 24 ± 2 | |
| 3 | 100 ± 6 | 45 ± 2 | |
| 4 | 80 ± 3 | 20 ± 2 | |
| 5 | 98 ± 3 | 55 ± 3 | |
| 6 | 106 ± 6 | | 36 ± 3 |
| 7 | 96 ± 5 | | 50 ± 3 |
| 8 | 102 ± 5 | | 35 ± 3 |
| 9 | 95 ± 3 | | 23 ± 3 |
| 10 | 90 ± 3 | | 42 ± 2 |
| Mean MMI ± S.E. | 95 ± 4 | 35 ± 7 | 38 ± 5 |

[a] Mean ± S.E.

Table 3

*Conversion of nonsensitive PEC to MOPC-315 TAAg responsiveness with RNA from 5- or 6-day tumor-bearing BALB/c mice*

All experiments were performed with pooled PEC from 10 nonsensitive BALB/c mice. RNA was obtained from mice grafted with 1 × $10^6$ MOPC-315 cells 5 or 6 days previously.

| Experiment | MMI of normal PEC (%) tested with | | | | |
|---|---|---|---|---|---|
| | 5- to 6-day I-RNA + MOPC-315 TAAg | 5- to 6-day I-RNA | MOPC-315 TAAg | Normal RNA + MOPC-315 TAAg | 5- to 6-day I-RNA + MOPC-104E Ag |
| 1 | 29 ± 1[a] | 102 ± 3 | 95 ± 1 | 88 ± 2 | ND[b] |
| 2 | 45 ± 1 | 78 ± 2 | 78 ± 4 | 75 ± 2 | ND |
| 3 | 46 ± 2 | 88 ± 5 | 81 ± 3 | ND | 95 ± 3 |
| 4 | 33 ± 2 | 83 ± 4 | 104 ± 2 | 88 ± 2 | ND |
| 5 | 43 ± 2 | 92 ± 6 | 80 ± 3 | ND | ND |
| 6 | 58 ± 3 | 121 ± 7 | 100 ± 4 | 102 ± 4 | 130 ± 5 |
| 7 | 41 ± 1 | 94 ± 5 | 90 ± 2 | ND | ND |
| Mean MMI ± S.E. | 40 ± 6 | 94 ± 5 | 89 ± 6 | 87 ± 5 | 112 ± 4 |

[a] Mean ± S.E.
[b] ND, not done.



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Table 4

*Conversion of unresponsive PEC from MOPC-315-bearing mice to MOPC-315 TAAg responsiveness with 5- or 6-day I-RNA*

All experiments were performed with pooled PEC from 10 mice. Donor PEC were obtained from mice grafted 14 days previously with 1 × 10^{6} MOPC-315 cells.

| | MMI of 14-day PEC (%) tested with[a] | | | | |
|---|---|---|---|---|---|
| Experiment | 5- to 6-day RNA + MOPC-315 TAAg | 5- to 6-day RNA | MOPC-315 TAAg | Normal RNA + MOPC-315 TAAg | 5- to 6-day RNA + MOPC-104E TAAg |
| 1 | 39 ± 2[b] | 94 ± 5 | 108 ± 6 | ND[c] | 98 ± 4 |
| 2 | 50 ± 1 | 102 ± 3 | 100 ± 4 | 109 ± 5 | ND |
| 3 | 36 ± 2 | 87 ± 2 | 88 ± 2 | ND | 112 ± 6 |
| 4 | 47 ± 3 | 112 ± 4 | 95 ± 3 | 90 ± 3 | 106 ± 3 |
| 5 | 45 ± 2 | 88 ± 3 | 116 ± 5 | ND | ND |
| 6 | 51 ± 3 | 101 ± 2 | | 88 ± 4 | ND |
| Mean MMI ± S.E. | 45 ± 1 | 97 ± 5 | 104 ± 6 | 95 ± 7 | 105 ± 5 |

[a] RNA (200 to 500 μg/2 to 5 × 10^{7} PEC) obtained from normal mice or mice grafted 5 or 6 days previously with 1 × 10^{6} MOPC-315 cells.

[b] Mean ± S.E.

[c] ND, not done.

Table 5

*MIF test for MOPC-315 TAAg responsiveness with nonsensitive PEC treated with RNA from 12- to 14-day tumor-bearing, or 12- to 14-day PPD-immune tumor-bearing mice*

All experiments were performed with pooled PEC from 10 mice.

| | MMI of normal mouse PEC (%) tested with | | | | |
|---|---|---|---|---|---|
| Experiment | 12- to 14-day RNA[a] + MOPC-315 TAAg | 12- to 14-day RNA[a] | MOPC-315 TAAg | Normal RNA + MOPC-315 TAAg | |
| 1 | 105 ± 4 | 100 ± 2 | 88 ± 4 | 90 ± 5 | |
| 2 | 86 ± 5 | 80 ± 2 | 78 ± 4 | 81 ± 4 | |
| 3 | 121 ± 7 | 92 ± 4 | 105 ± 6 | N.D. | |
| 4 | 80 ± 3 | 102 ± 5 | 83 ± 3 | 103 ± 3 | |
| 5 | 118 ± 6 | 130 ± 5 | 100 ± 4 | N.D. | |
| 6 | 105 ± 5 | 112 ± 4 | 103 ± 5 | 90 ± 3 | |
| 7 | 78 ± 2 | 71 ± 2 | 77 ± 2 | 72 ± 2 | |
| Mean MMI ± S.E. | 98 ± 7 | 99 ± 7 | 91 ± 5 | 91 ± 4 | |
| Experiment | 12- to 14-day PPD[b] RNA + MOPC-315 TAAg (%) | 12- to 14-day PPD-RNA (%) | MOPC-315 TAAg (%) | PPD (%) | 12- to 14-day PPD-RNA[b] + PPD (%) |
| 8 | 112 ± 6 | 100 ± 9 | 125 ± 10 | 79 ± 5 | 36 ± 4 |
| 9 | 103 ± 8 | 111 ± 8 | 116 ± 8 | 78 ± 5 | 45 ± 3 |

[a] RNA (200 to 500 μg/2 to 5 × 10^{7} PEC) was obtained from mice grafted 12 to 14 days previously with 1 × 10^{6} MOPC-315 cells.

[b] RNA (200 to 500 μg/2 to 5 × 10^{7} PEC) was obtained from mice grafted 12 to 14 days previously with 1 × 10^{6} MOPC-315 cells and immunized 10 to 12 days previously with 100 μg mycobacteria as described in text.

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mice were grafted with tumor cells and immunized with mycobacteria 2 days later. Fourteen days posttumor grafting, the mice were sacrificed and RNA was prepared from their lymphoid tissues. When nonsensitive PEC were treated with this RNA (PPD-RNA) and stimulated with PPD, the cells were inhibited in their migration relative to the controls; the average MMI was 41% (Table 5).

**Failure of 12- to 14-Day RNA to Abrogate MIF Production in PEC from 6-Day Postgrafting Responsive Tumor-bearing Mice.** As a test of whether 12- to 14-day RNA is capable of blocking, suppressing, or altering MOPC-315 tumor antigen reactivity in responsive PEC populations, PEC from mice grafted 6 days previously with 1 × 10^{6} MOPC-315 cells were treated with 12- to 14-day RNA and tested for MIF production upon stimulation with soluble MOPC-315 antigen. When 6-day responsive cells were treated with 12- to 14-day RNA and incubated in medium alone, the cells migrated comparably to the controls; the average MMI was 96% (Table 6). On the other hand, when 6-day cells were treated with 12- to 14-day RNA and stimulated with MOPC-315 TAAg, the cells retained their capacity to elaborate MIF; the average MMI was 48% (Table 6). Similarly, when

these PEC were not treated with RNA but were stimulated with MOPC-315 TAAg, inhibition of migration was demonstrated; the average MMI was also 48% (Table 6). Thus, 12- to 14-day RNA is not capable of abrogating the tumor antigen reactivity of 6-day responsive cells and therefore is not responsible for the depression of MOPC-315 immunity seen by 10 to 14 days of tumor growth in MOPC-315-bearing animals. A summary of Tables 3 to 6 is shown in Chart 2.

**Sucrose Density Gradient Analysis.** Sucrose density gradient analysis was performed with RNA used in these experiments to ensure that the RNA was intact prior to testing its activity *in vitro*. When 5- to 6-day I-RNA preparations exhibited 4 S peaks only, or when RNAse-treated preparations were tested, the capacity of 5- to 6-day I-RNA to transfer MOPC-315 immunity was abolished.

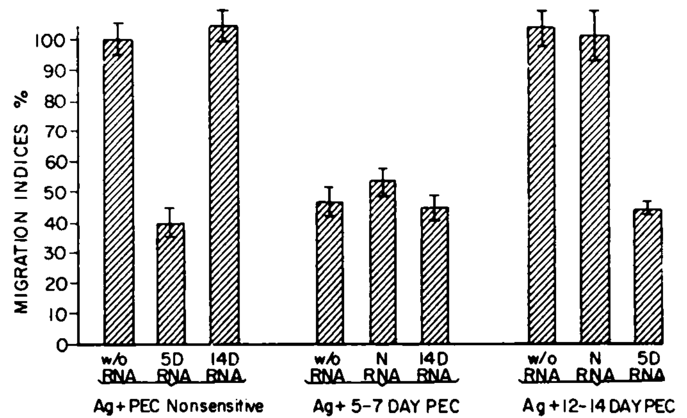


Chart 2. Effect of normal RNA or RNA from MOPC-315-bearing animals on the migration of PEC obtained from normal or tumor-bearing mice in the presence or absence of antigenic stimulation with MOPC-315 TAAg. *N RNA*, RNA from normal BALB/c mice. *5 D RNA*, RNA from mice grafted 5 or 6 days previously with 1 × $10^6$ MOPC-315 tumor cells. *14 D RNA*, RNA from mice grafted 12 to 14 days previously with 1 × $10^6$ MOPC-315 cells. *Bars*, S.E.

## DISCUSSION

Mice bearing a lethal plasmacytoma mounted a transient, tumor-specific, cell-mediated immune response early during lethal tumor growth. The disappearance of detectable antitumor antigen reactivity in later stages of tumor development did not lead to a concomitant loss of nontumor cellular immunity in the host. RNA-rich extracts from spleens of tumor-bearing mice at 5 and 6 days of tumor development were able to transfer *in vitro* tumor antigen responsiveness to nonsensitive PEC or PEC from terminal unresponsive tumor-bearing hosts. Conversely, RNA extracts from spleens of terminal MOPC-315 bearers were unable to transfer tumor antigen responsiveness to nonsensitive mouse PEC and were unable to suppress tumor antigen reactivity of responsive cells.

Mice immunized to plasmacytoma tumors exhibit cell-mediated immunity to plasmacytoma-associated antigens as shown by the MIF test (19) and immunoprotection tests (13, 20). The data in this report extends these findings by demonstrating the development of cell-mediated reactivity to plasmacytoma antigens early during tumor growth in mice bearing lethal tumors. The cell-mediated antitumor reactivity of MOPC-315-bearing mice appeared to be specific for MOPC-315 tumor antigens, although a recent report by Burton and Warner (3) indicates that plasmacytoma share some tumor-associated antigens. The presence of a lethal plasmacytoma did not abrogate the cell-mediated immune response of the host to nontumor antigens since MOPC-315-bearing animals immunized with *M. tuberculosis* or DNP-Ig retained cellular immunity to the immunizing antigen after losing responsiveness to tumor antigens. This finding is similar to reports by Zolla-Pazner *et al.* (24) who found that plasmacytomatous mice retain the capacity to mount cell-mediated responses to allogeneic cells, mitogens, and dinitrochlorobenzene. Thus, the apparent response of mice to a progressively growing plasmacytoma is to mount cell-mediated immunity to tumor antigens early after tumor challenge and subsequently to lose immunity to the tumor by a mechanism that does not depress nontumor cell-mediated reactions.

The capacity of RNA-rich extracts from tumor-immune animals to transfer tumor antigen reactivity to nonsensitive lymphoid cells has been demonstrated in several laboratories (4, 12, 16–18). Less is known, however, about the activity of I-RNA prepared from the tissues of tumor-bearing animals. Kern *et al.* (9) have studied the kinetics of synthesis of antitumor I-RNA in rats bearing progressively growing MC3-R tumors and reported that the antitumor activity of the I-RNA was maximal between 21 and 28 days postgrafting; little antitumor activity was detectable in I-RNA prepared during the 1st 7 days of MC3-R tumor growth. Our data extend to the MOPC-315 plasmacytoma system the observation that I-RNA can be obtained during some, but not all, stages of tumor growth in animals bearing progressively growing tumors. Furthermore, our data show a correlation between the antitumor activity of tumor-bearer I-RNA and the tumor antigen reactivity of tumor-bearer PEC. It is conceivable that the factors responsible for the apparent loss of tumor antigen responsiveness in 12- to 14-day PEC might also affect I-RNA synthesis. However, the factors that regulate the rapid appearance and apparent disappearance of antitumor I-RNA during progressive MOPC-315 growth are not known at present.

The potential usefulness of I-RNA for human immunotherapy depends in part on being able to sensitize lymphocytes from tumor-bearing individuals whose cells may be suppressed for tumor immunity (5). Since the kinetic study of

Table 6

*MIF test for MOPC-315 TAAg responsiveness with PEC from 6-day tumor-bearing mice treated with RNA from 12- to 14-day tumor-bearing mice*

All experiments were performed with pooled PEC from 10 mice. PEC were from mice grafted 6 days previously with 1 × $10^6$ MOPC-315 cells. RNA (200 to 500 $\mu$g/2 to 5 × $10^7$ PEC) were obtained from mice grafted 12 to 14 days previously with 1 × $10^6$ MOPC-315 cells.

| Expt. | 12- to 14-day RNA + MOPC-315 TAAg (%) | 12- to 14-day RNA (%) | MOPC-315 TAAg (%) |
|---|---|---|---|
| 1 | 46 ± 3 | 109 ± 5 | 48 ± 4 |
| 2 | 58 ± 4 | 89 ± 4 | 55 ± 4 |
| 3 | 40 ± 4 | 90 ± 3 | 42 ± 3 |
| 4 | 48 ± 5 | 96 ± 6 | 48 ± 5 |
| Mean MMI ± S.E. | 48 ± 4 | 96 ± 5 | 48 ± 3 |



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MOPC-315-bearing mice revealed that tumor antigen reactivity in PEC of the host becomes undetectable during tumor development, this system is a useful model to study the effects of I-RNA transfer to cells from unresponsive tumor-bearing hosts. The results of these experiments reveal that I-RNA from responsive tumor bearers is capable of transferring tumor antigen reactivity to unresponsive lymphoid cells of the tumor-bearing host. The PEC from terminal MOPC-315-bearing mice that were converted to a responsive state by I-RNA may be unresponsive due to *(a)* blocking by humoral factors as suggested by Kolb *et al.* (10) and Braun and Dray (2), *(b)* suppression by other lymphoid elements, *(c)* suppression by tumor cell factors, or *(d)* depletion of tumor-reactive cells from the peritoneal cavity. Thus, the action of I-RNA on the unresponsive PEC population might be envisaged to transfer tumor antigen reactivity to unblocked, nonsensitive cells within the population or to derepress blocked, tumor-reactive cells. The data do not elucidate the nature of the cells in the unresponsive PEC population that are the targets for I-RNA transfer of tumor antigen reactivity.

We have shown that autochthonous I-RNA may not be available from a tumor-bearing host at some stages of tumor growth. Therefore, xenogeneic and allogeneic sources of I-RNA should be tested. Since xenogeneic and allogeneic I-RNA have been shown to transfer cell-mediated immunity to nonsensitive lymphoid cells, we would expect that they might also transfer tumor immunity to "suppressed" lymphoid populations from tumor-bearing hosts.

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