AUGMENTATION OF MOUSE NATURAL KILLER ACTIVITY AND INDUCTION OF INTERFERON BY TUMOR CELLS IN VIVO

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Natural killer (NK)[1] cells, a subpopulation of lymphocytes with the ability to lyse tumor cells, have been demonstrated in a variety of experimental animal species and in man (1, 2). One of the characteristics of NK-cell activity in mice and rats is that it is first detectable at 3–4 wk of age, reaches a peak at 5–8 wk, and declines thereafter to low or undetectable levels. Some insight into the factors regulating the expression of NK activity has been provided by the observation that inoculation of tumor cells (3), viruses (3–5), and bacterial products (3, 6) into mice led to augmentation or reappearance of NK activity within 3 d. A large number of the materials that augmented NK cytotoxicity are known to be able to induce interferon (IF), and the possibility that IF mediated the boosting of NK activity was therefore considered. Indeed, direct evidence is now available for the boosting of NK activity in vivo and in vitro by type I IF that has been induced by Newcastle disease virus (NDV) and by other common IF inducers (7, 8).

The mechanism for augmentation of NK activity by inoculation of tumor cells is less clear. Trinchieri et al. (9) recently reported that human lymphocytes cultured in vitro with tumor cells for 18–24 h produced soluble factors that can augment NK activity as well as display antiviral properties. In the light of these findings, we initiated the present study to determine whether in vivo inoculation of tumor cells that led to increased NK activity (3) could also induce detectable circulating levels of IF. We have found that several syngeneic and allogeneic tumor cells induced remarkably high serum levels of IF and that the kinetics of IF induction was similar to that of boosting of NK activity in the spleen. The IF induced by tumor cells in vivo was acid labile and required prolonged incubation with L cells to induce full protection against virus challenge, thus resembling type II IF (10).

Materials and Methods

Mice. Female BALB/c, C57BL/6, CBA/N, and DBA/2 mice, as well as BALB/c nude mice, were obtained from the Rodent and Rabbit Production Section, Division of Research Services, National Institutes of Health, Bethesda, Md.

[1] *Abbreviations used in this paper:* IF, interferon(s); LCMV, lymphocytic choriomeningitis virus; MLV, Moloney leukemia virus; MSV, murine sarcoma virus; NDV, Newcastle disease virus; NK, natural killer; RLV, Rauscher leukemia virus.

Cell Lines. Most of the in vivo and in vitro tumor cell lines have previously been described (11). Ascitic and tissue culture tumor lines are designated by the suffixes A and TC, respectively. The BALB/c-transformed cell line, WM-7, derived from a spontaneous fibrosarcoma and the same cell line infected with Rauscher leukemia virus, R-WM-7, were provided by Dr. I. Al-Ghazzouli, Microbiological Associates, Walkersville, Md. (12).

Viruses. Murine sarcoma virus (MSV), Moloney leukemia virus (MLV), and Rauscher leukemia virus (RLV) were obtained from the Virus Cancer Program of the National Cancer Institute (Bethesda, Md.) through the courtesy of Dr. Jack Gruber. Ter A type C virus, shed from a spontaneous C3H/HeICRF ovarian teratoma cell line, was provided by Dr. W. J. Martin, Food and Drug Administration, Bureau of Biologics, Bethesda, Md. (13). Lymphocytic choriomeningitis virus (LCMV), attenuated Armstrong strain CA1371, was provided by Dr. W. Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md., and influenza virus, strain A/Port Chalmers, was obtained from Dr. F. A. Ennis, Food and Drug Administration, Bureau of Biologics. NDV was prepared in fertile chick eggs as previously described (14).

Antibodies to IF. Two sources of anti-mouse type I IF globulin were used in this study. One was the gift of Dr. I. Gresser, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France, and its specificity has been described in detail elsewhere (7). The other anti-IF globulin was provided by Dr. K. Paucker, The Medical College of Pennsylvania, Philadelphia, Pa. Both reagents were produced against purified mouse IF induced in L cells by NDV.

Virus-induced Serum IF. BALB/c mice, inoculated with NDV, were bled for serum IF 10 h later, as previously described (14).

Inhibition of Protein Synthesis. RL♂1 ascites tumor cells were incubated with a 10^{-5} M solution of pactamycin (15) for 45 min at 37°C. The cells were then washed twice with medium before inoculation into mice.

In Vivo Challenge with Tumor Cells or Viruses. Tumor cells or viruses detected in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) were inoculated intraperitoneally in RPMI-1640 medium. Each mouse received 1×10^7 viable tumor cells or 0.2 ml of a stock solution of virus. At various intervals after inoculation, three mice per group were bled and their pooled sera were tested for circulating levels of IF. The spleen cells from the same mice were also pooled and tested for NK activity.

Titration of IF. Antiviral activity in the serum samples was measured by the degree of inhibition of the cytopathic effect caused by vesicular stomatitis virus (14).

Measurement of NK Activity. Spleen cells, pooled from three mice, were incubated at 200:1 and 50:1 ratios with ^{51}Cr-labeled RL♂1 tumor target cells for 4 h at 37°C, as previously described (7). Cryopreserved target cells were used to minimize day-to-day variation in the assay and the base-line ^{51}Cr release was determined by the use of unlabeled autologous tumor cells in place of lymphoid cells. By using a combination of cryopreserved tumor cells and autologous control cultures, the base-line lysis was consistently between 5 and 8% and the median standard error was 0.6%. Levels of cytotoxicity of 2% or more above the base line consistently were significantly different from the controls by the Student's t test ($P < 0.05$).

The percent cytotoxicity was calculated as follows:

$$\frac{\text{cpm released from experimental group} - \text{cpm released from autologous control} \times 100}{\text{total cpm incorporated.}}$$

In all the tables, only the 200:1 ratio was reported because the lower percent cytotoxicity was dose responsive and the lower ratio yielded a similar pattern of results.

Results

In Vivo Boosting of NK Activity and Induction of IF by Mouse Tumor Cells. 12-wk-old BALB/c and BALB/c nude mice, expected to have low levels of spontaneous NK activity, were inoculated intraperitoneally with either RL♂1, RBL-5, or EL-4 G+ ascites tumor cells. At various times after inoculation, sera were obtained to test for the presence of circulating IF, and the spleen cells were tested for NK activity. Three representative experiments are shown in Table I. All of these cell lines were capable

TABLE I

Boosting of NK Activity and Induction of IF by Mouse Tumor Cells

Experiment	Recipient	Inoculum*	Strain of origin	Percent cytotoxicity (units of IF/ml serum)		
				1 d	2 d	3 d
1	BALB/c	None		3.3 (<5)	2.9 (<5)	4.1 (<5)
		Medium		2.4 (<5)	2.6 (<5)	4.6 (<5)
		RL♂1 A	BALB/c	18.4 (398)	15.8 (1,995)	5.9 (<5)
		RBL-5 A	C57BL/6	18.7 (501)	17.6 (1,000)	5.2 (<5)
		EL-4 G+ A	C57BL/6	15.9 (126)	18.8 (1,259)	16.1 (133)
2	BALB/c	None		1.8 (<5)	2.4 (<5)	2.6 (<5)
	Nude	Medium		1.8 (<5)	2.4 (<5)	2.8 (<5)
		RL♂1 A	BALB/c	24.5 (2,511)	19.4 (1,258)	4.8 (<5)
		RBL-5 A	C57BL/6	16.8 (3,981)	25.4 (3,162)	12.9 (<5)
		EL-4 G+ A	C57BL/6	20.1 (630)	18.3 (1,995)	3.5 (<5)
3	BALB/c	None		4.5 (<5)	3.6 (<5)	3.3 (<5)
		Medium		3.6 (<5)	2.7 (<5)	4.0 (<5)
		RL♂1 A	BALB/c	23.8 (6,310)	26.9 (3,162)	18.3 (10)
		RL♂1 A (pact.)‡	BALB/c	19.2 (6,310)	22.3 (1,000)	
		RL♂1 TC	BALB/c	4.1 (<5)	21.7 (1,000)	
		RBL-5 A	C57BL/6	21.2 (50,118)	20.1 (6,301)	5.7 (32)
		RBL-5 TC	C57BL/6	18.7 (3,981)	15.6 (3,162)	

* 10^7 tumor cells were inoculated intraperitoneally into 12-wk-old mice.

‡ RL♂1 tumor cells were incubated with 10^{-5} M of pactamycin for 45 min at 37°C and washed before inoculation into mice.

of augmenting NK activity in both conventional and nude mice, and substantial increases were seen within 1 d of challenge. The activity was transient and usually declined or returned to base-line levels by day 3. Serum antiviral activity was also induced by these tumor-cell lines and often reached remarkably high levels. For each cell line, there was a close correlation between the kinetics of augmentation of NK activity and the kinetics of induction of IF. The tissue culture lines of RL♂1 and RBL-5 tumor cells induced similar levels of NK activity and IF as the ascitic lines.

Because the tumor cells used in these experiments were lymphomas that might have the ability to produce lymphokines themselves, it was important to rule out that possibility. Therefore, mice were inoculated with RL♂1 tumor cells that had been pretreated with pactamycin for 45 min at 37°C to inhibit protein synthesis (Table I). This treatment, which has been shown to completely and irreversibly block protein synthesis, had no adverse effect on the ability of RL♂1 tumor cells to augment NK activity or to induce IF. The IF thus appeared to be host derived.

The kinetics of tumor cell-induced augmentation of NK activity and induction of circulating levels of IF were then more closely examined. As seen in a representative experiment in Table II, 56 U of IF/ml were already detectable in the serum at 4 h after challenge with RL♂1 tumor cells but no significant rise in NK cells was seen. The two activities rose steadily thereafter to peak at 15–24 h. By day 3, the IF level had declined and the NK activity had correspondingly decreased, and both activities approached background levels by day 6.

Challenge of BALB/c Mice with a Variety of Tumor and Normal Cells. To further investigate the parallelism between boosting of NK cells and IF production, BALB/c

TABLE II

Early Detection of NK Cells and Circulating IF after RLδ1 Tumor Cell Inoculation

Time after RLδ1 inoculation*	Percent cytotoxicity (units of IF/ml serum)
0 h	2.8 (<5)
4 h	3.4 (56)
8 h	10.5 (177)
15 h	27.2 (3,162)
24 h	26.2 (5,623)
3 d	15.6 (446)
6 d	5.0 (<5)

* 10^7 ascites tumor cells were inoculated intraperitoneally into 12-wk-old BALB/c mice.

TABLE III

Boosting of NK Activity and Induction of IF by Various Tumor and Normal Cells

Inoculum*	Strain of origin	Percent cytotoxicity (units of IF/ml serum)	
		1 d	3 d
None		2.3 (<5)	0.8 (<5)
LSTRA A	BALB/c	20.4 (1,000)	16.7 (1,259)
L1210 A	DBA/2	16.6 (2,511)	9.7 (631)
P815 A	DBA/2	19.7 (1,000)	10.1 (25)
YAC TC	A/Sn	21.4 (478)	5.6 (<5)
WM-7 TC	BALB/c	2.9 (<5)	1.8 (<5)
R-WM-7 TC	BALB/c	3.9 (<5)	2.1 (<5)
L929 TC	C3H	2.5 (<5)	2.2 (<5)
Thymus cells	CBA/N	8.2 (5)	3.1 (5)
Spleen cells	CBA/N	2.0 (<5)	2.4 (<5)
Thymus cells	C57BL/6	10.1 (64)	6.3 (<5)
Spleen cells	C57BL/6	2.7 (<5)	2.4 (<5)

* 10^7 cells were inoculated intraperitoneally into 12-wk-old BALB/c mice.

mice were inoculated with a variety of syngeneic and allogeneic tumor or normal cells. One of four experiments performed is shown in Table III. Most of the tumor-cell lines activated the two activities within 1–3 d; however, WM-7, R-WM-7, and L 929 cells did not. As previously described (3), allogeneic thymus cells consistently caused a small rise in NK activity. This was accompanied by a low but detectable rise in IF. Allogeneic spleen cells, on the other hand, had no stimulatory effect on NK activity or IF levels. Although there was not a good quantitative relationship between the degree of NK boosting and IF level produced by the various inocula, all cells capable of boosting NK activity also induced some increase in serum IF.

Challenge with Viruses. To ascertain whether type C viruses that often contaminate tumor-cell lines might be responsible for the observed activation of NK cells and induction of IF, BALB/c mice were inoculated with MSV, MLV, RLV, or Ter A virus and then tested for four consecutive days (Table IV). For comparison, the nononcogenic viruses, LCMV and influenza A, were also studied. MSV augmented

TABLE IV

Effect of Various Viruses on Boosting of NK Activity and on Induction of IF

Inoculum*	Percent cytotoxicity (units of IF/ml serum)				
	1 d	2 d	3 d	4 d	6 d
None	12.1 (<10)	7.4 (<10)	10.1 (<10)	12.8 (<10)	9.2 (<10)
Tumor cells					
RLδ1 A	25.0 (1,584)	25.8 (11,481)	15.2 (501)	14.3 (10)	8.7 (<10)
RBL-5 A	16.4 (5,011)	20.4 (3,981)			
Type C viruses					
MSV	25.3 (891)	17.3 (49)	14.7 (<10)	9.7 (<10)	
MLV	12.0 (<10)	10.3 (<10)	11.0 (<10)	13.9 (<10)	
RLV	10.3 (<10)	8.8 (<10)	5.8 (<10)	14.2 (<10)	
Ter A	9.4 (<10)	7.1 (<10)	9.2 (<10)	10.1 (<10)	
Other viruses					
LCMV	8.8 (<10)	6.5 (10)	30.9 (158)	31.3 (25)	10.7 (10)
Influenza A	6.8 (<10)	10.4 (<10)	27.7 (10)	37.7 (64)	17.1 (10)

* 10^7 tumor cells or 0.2 ml of a stock solution of virus were inoculated intraperitoneally into 12-wk-old BALB/c mice.

TABLE V

Comparison of the Characteristics of Tumor-induced IF with NDV-induced IF

Experiment	Treatment	Units of IF/ml serum		
		NDV-IF	RLδ1-IF	RBL-5-IF
1	None	56,234	5,623	6,309
	56°C, 1h	<10	316	251
	Anti-viral activity with HeLa cells	<10	<10	<10
	pH 3.0	50,118	<10	<10
	pH 6.0		4,778	5,523
	pH 9.0		5,011	4,786
	Incubation with IF*			
	1 h	50,118	<10	<10
	4 h	50,118	398	2,511
	8 h		5,011	5,523
	12 h		5,011	6,309
2	None	63,095	1,000	
	Normal globulin			
	1:400	60,200	794	
	Anti-IF (Paucker)			
	1:400	<10	<10	
	1:800	<10	<10	
	None	17,782		7,585
	Normal globulin			
	10^{-4}	17,782		6,855
	10^{-5}	17,782		6,855
	10^{-6}	18,000		7,079
	Anti-IF (Gresser)			
	10^{-4}	<10		<10
	10^{-5}	<10		<10
	10^{-6}	3,981		<10

* L 929 cells were incubated with various dilutions of each IF for the time period indicated and then washed twice before they were exposed to virus challenge.

NK activity and induced serum IF. However, as previously described, other type C viruses had no effect on the two activities. LCMV and influenza viruses readily activated the two functions, but with a later response than that seen with MSV or tumor cells. Both activities peaked at day 3 after virus challenge and peak levels of IF were much lower than those seen after tumor challenge.

Characteristics of Tumor-induced IF. The characteristics of the antiviral activity in the serum samples collected 24 h after RL♂1 or RBL-5 tumor challenge were compared with those of type I IF induced in vivo by NDV (Table V). The virus-induced and tumor-induced IF lost appreciable activity after incubation for 56°C for 1 h, and both preparations exhibited species specificity in their inability to protect human HeLa cells from virus challenge. However, the two types of IF specimens differed in other respects. Unlike virus-induced IF, the tumor-induced IF was labile at pH 3.0. In addition, preincubation with virus-induced IF for 1 h fully protected L 929 cells against VSV, but a considerably longer incubation period was required for tumor-induced IF. Although the pH lability of the tumor-induced IF and the need for prolonged incubation to induce viral resistance were characteristic of type II IF (10), the antiviral activity of the tumor-induced IF was readily neutralized by antisera to virus-induced IF obtained from two sources. This led us to consider the possibility that the samples collected at 24 h might contain a mixture of virus-induced and tumor-induced IF. Samples taken at various times after RL♂1 tumor inoculation between 4 and 48 h were therefore tested for acid and heat lability as well as sensitivity to antivirus-induced IF. All the samples were found to have the same properties as listed above, which made it likely that the anti-IF are not type specific.

Discussion

Our study clearly indicates that tumor cells in addition to other inocula that have previously been shown to boost NK activity (3–8), can induce high levels of circulating IF in vivo. This is analogous to the findings of Trinchieri et al. (9) who detected antiviral activity in the supernates of human lymphocytes cultured in the presence of tumor cells. These observations are consistent with the hypothesis that augmentation of NK activity, regardless of the type of stimulus, is mediated by induction of IF.

The appearance of circulating IF coincided well with the development of NK activity in tumor-inoculated mice. Both activities peaked at 24–48 h and usually declined by 3 d. In contrast, peak activities after virus challenge were seen only after 3 d, and base-line levels were reached after 6 d. The kinetics of response to tumor cells were, in fact, earlier than those previously reported from our laboratory (3), and the differences in kinetics are not yet clear. The previous study was performed with irradiated tumor cells and it is possible that this altered the kinetics. The health of the mice and their previous exposure to various IF inducers, such as viruses, may also affect the kinetics. Other investigators have observed that tumor cells can induce IF in vivo. The kinetics of IF production as reported by Svet-Moldavsky et al. (16) and Skurkovich et al. (17) were very similar to those reported here. Svet-Moldavsky et al. (18), however, detected a small rise in circulating IF after intravenous transfer of allogeneic spleen cells, which differs from our data. This may be a result of the route of inoculation, because these authors observed a difference in the level of IF production by intravenous and intraperitoneal inoculation. We found that allogeneic thymus

cells caused some rise in NK cells and IF, but both levels were much lower than those observed with tumor cells.

The antiviral activity in the serum of mice inoculated with tumor cells met several criteria for mouse IF. It exhibited heat lability, had the ability to stimulate viral resistance in indicator L cells, had species specificity, and was neutralized by anti-IF globulin. The IF induced by tumor cells resembled type II IF in being acid labile. In addition, it required 8 h of incubation with L cells to induce full protection similar to type II IF induced by staphylococcal enterotoxin A (10).

The possibility that IF induction and NK boosting by tumor cells might be a result of virus contamination of the tumor lines was evaluated. Because a variety of nononcogenic viruses that are frequent contaminants of mice and tumor-cell lines have been shown to boost NK function (3), probably by their ability to induce IF, this is of particular concern. In addition, most of the tumor-cell lines express type C viruses and these might cause the observed effects. This latter possibility is unlikely because our previous (3) and present findings showed that a variety of type C viruses failed to boost NK cells or induce IF. MSV type C virus was the only exception, but it is possible that the stimulatory effect of this virus has been a result of the use of a crude preparation that was made from a pool of solid tumors that was simply homogenized and centrifuged to remove most cell debris. It is thus quite likely that the MSV preparation contained tumor antigens and other cell contaminants. It is also possible that MSV has the ability to rapidly transform cells that could then boost NK activity. Still another possibility is that it is contaminated with a nononcogenic virus. Nononcogenic viruses, however, induced NK cells and IF in vivo with kinetics distinctly separate from those induced by MSV or tumor cells. Moreover, the tumor-induced IF showed antiviral properties resembling type II IF rather than type I IF. Interestingly enough, the IF induced by in vitro culture of human lymphocytes with tumor cells was found to have characteristics largely of type I IF as characterized by gel filtration and acid stability (9).

The cell type that is responsible for the production of IF is not yet known. Trinchieri et al. (9), presented some evidence for IF production by NK cells themselves, in response to tumor cells in vitro. However, macrophages appeared to be the main source of IF (19) by poly inosinic:poly cytidylic acid boosting of mouse NK activity. Similarly, macrophages are required in the Bacille Calmette-Guérin boosting of NK activity (6). In this study, we found that nude mice could respond to tumor cells in vivo by high levels of IF production, which suggests that mature T cells were not involved.

The finding that all of the tumor cell-induced IF activity was acid labile and that it required prolonged binding to indicator cells for full protection indicates that type II mouse IF also has the ability to strongly boost NK activity. These data suggest that one of the earliest events triggered in the host by recognition of tumor cells is the production of immune IF that has the ability to promote lysis of the relevant target cells. Indeed, many of the tumor cells, including thymus cells, that are capable of boosting NK activity and inducing IF are sensitive to NK lysis, and it is possible that the boosting of the two activities is a result of rapid and specific recognition of NK target-cell structures. It is reasonable to interpret these data by assuming that the NK cells themselves may be responsible for IF production, and it will be important to directly examine this point. This postulated sequence of events support a potential

role of NK cells in natural and nonthymus-dependent defenses against tumors, even when spontaneous levels of NK activity are low. Recognition of NK-sensitive tumor cells could, within hours, result in appreciable levels of antitumor activity in the host.

Summary

Conventional and nude mice inoculated with syngeneic or allogenic tumor cells developed a rapid rise in serum interferon (IF) levels, peaking within 24 h. Within the same period, natural killer (NK) activity was readily boosted in the spleen. Both activities usually declined at 3 d. Cells that lacked the ability to augment NK activity also failed to induce detectable levels of IF. The boosting of IF and NK functions did not appear to be a result of contamination of the tumor lines by viruses because inoculation of several type C viruses into normal mice had no effect, and other viruses, like lymphocytic choriomeningitis virus and influenza, elevated IF and NK levels with a significantly later kinetics, peaking 3–4 d. The IF induced by tumor cells was heat and acid labile, species specific, and appeared to be in the type II class, although it was susceptible to antisera against Newcastle disease virus-induced IF. These data suggest that an early, nonthymus-dependent consequence of tumor-cell recognition is the production of IF, which, in turn, activates NK cells to lyse the tumor cells.

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References

1. Herberman, R. B., and H. T. Holden. 1978. Natural cell-mediated immunity. *Adv. Cancer Res.* **27:**305.
2. Cudkowicz, G., and P. S. Hochman. 1979. Do natural killer cells engage in regulated reaction against self to ensure homeostasis? *Immunol. Rev.* **44:**71.
3. Herberman, R. B., M. E. Nunn, H. T. Holden, S. Staal, and J. Y. Djeu. 1977. Augmentation of natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic target cells. *Int. J. Cancer.* **19:**555.
4. MacFarlan, R. I., W. H. Burns, and D. O. White. 1977. Two cytotoxic cells in peritoneal cavity of virus-infected mice: antibody-dependent macrophages and non-specific killer cells. *J. Immunol.* **119:**1569.
5. Welsh, R. M., and R. M. Zinkernagel. 1977. Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice. *Nature (Lond.).* **268:**646.
6. Tracy, D. E. 1979. The requirement for macrophages in the augmentation of NK cell activity by BCG. *J. Immunol.* **123:**840.
7. Gidlund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser. 1978. Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature (Lond.).* **273:**759.
8. Djeu, J. Y., J. A. Heinbaugh, H. T. Holden, and R. B. Herberman. 1979. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. *J. Immunol.* **122:** 175.
9. Trinchieri, G., D. Santoli, R. R. Dee, and R. B. Knowles. 1978. Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the antiviral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. Exp. Med.* **147:**1299.
10. Dianzani, F., L. Salter, W. R. Fleischmann, Jr., and M. Zucca. 1978. Immune interferon activates cells more slowly than does virus-induced interferon. *Proc. Soc. Exp. Biol. Med.* **159:** 94.
11. Herberman, R. B., T. Aoki, M. Nunn, D. H. Lavrin, N. Soares, A. Gazdar, H. Holden, and

K. S. Chang. 1974. Specificity of ^{51}Cr release cytotoxicity of lymphocytes immune to murine sarcoma virus. *J. Natl. Cancer Inst.* **53:**1103.

12. Al-Ghazzouli, I. K., R. M. Donahoe, K. Y. Huang, B. Sass, R. Peters, and G. J. Kelloff. 1976. Immunity to virus-free syngeneic tumor cell transplantation in the BALB/c mouse after immunization with homologous tumor cells infected with type C virus. *J. Immunol.* **117:**2239.
13. Martin, S. E., and W. J. Martin. 1975. Naturally occurring cytotoxic tumor reactive antibodies directed against type C viral envelope antigens. *Nature (Lond.).* **256:**498.
14. Huang, K. Y., R. M. Donahoe, F. B. Gordon, and H. R. Dresser. 1971. Enhancement of phagocytosis by interferon-containing preparations. *Infect. Immun.* **4:**581.
15. Thorn, R. M., and C. S. Henney. 1976. Studies on the mechanism of lymphocyte mediated cytolysis. VI. A reappraisal of the requirement for protein synthesis during T cell mediated lysis. *J. Immunol.* **116:**146.
16. Svet-Moldavsky, G. J., E. G. Slavina, I. L. Leipunskaya, B. M. Nemirovskaya, S. N. Zinzar, and L. F. Morozova. 1975. Interferon, immunologic recognition and cytotoxicity of lymphoid cells. II. Specific inhibition with alloantiserum; interferon production after syngeneic tumor cell inoculation. *Interferon Scientific Memoranda.* **I:**A144.
17. Skurkovich, S. V., E. I. Eremkina, and A. S. Skorikova. 1978. The induction of interferon by leukemia cells in mice. *Interferon Scientific Memoranda.* **I:**A668/1.
18. Svet-Moldavsky, G. J., B. M. Nemirovskaya, T. V. Osipova, E. G. Slavina, S. N. Zinzar, N. V. Karmanova, and L. F. Morozova. 1974. Interferongenicity of antigens and early cytotoxicity of lymphocytes. *Folia Biol. (Prague).* **20:**225.
19. Djeu, J. Y., J. A. Heinbaugh, H. T. Holden, and R. B. Herberman. 1979. Role of macrophages in the augmentation of mouse natural killer cell activity by poly I:C and interferon. *J. Immunol.* **122:**182.