Rauscher virus Mice
Interferon Immune response

VITAMIN C, INTERFERON AND THE IMMUNE RESPONSE

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Summary: The role of vitamin C in its suggested protective participation against viral infection, and possibly against malignancy, may be multifactorial. Enhancement of interferon synthesis, augmentation of T-lymphocyte activity, and concomitant macrophage activation may all contribute to this protective role. In the studies reported, inclusion of vitamin C in the drinking water of mice resulted in increased levels of circulating interferon and associated amelioration of symptoms with murine leukemia virus administration. Humoral antibody responses were unaffected by ascorbate, whereas the cell-mediated immune response was elevated. Additionally, tissue culture studies have demonstrated significantly enhanced production of interferon in the presence of vitamin C.

The mechanism of action of ascorbic acid in its proposed protection against some viral infections, such as the common cold in humans, is not well understood. The possibility of its role in viral inactivation[1], as immunologic enhancer[2], and in interferon stimulation[3] has been variously postulated. With regard to the latter, induced or exogenously administered interferon has been reported to bring about transient remission in patients with acute leukemia, and has been demonstrated to be experimentally efficacious in altering leukemia of viral etiology in mice.

Interferons are a class of proteins capable of inhibiting virus replication in vertebrate cells. They are of cellular origin and are induced in the human and animal host in response to infections by many viruses and other intracellular parasites. A fairly wide range of materials including synthetic polynucleotides can stimulate the production of interferon. Interfer-

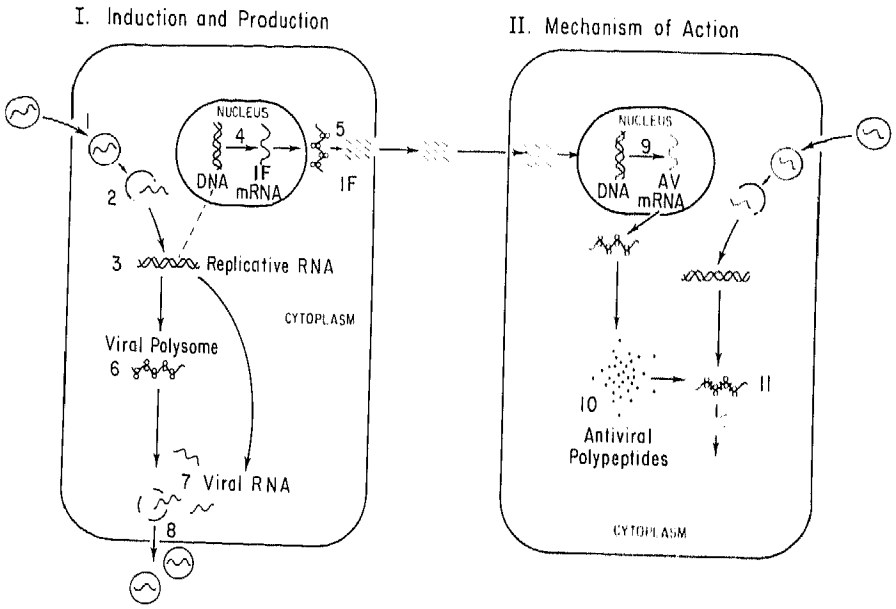

Fig. 1. Diagrammatic presentation of recent concepts in the synthesis and mechanism of action of interferon. The inducer example shown here is that of a single-stranded RNA virus, such as employed in the present (Rauscher virus) experiments. (I) Following attachment and entry of virus into the cell (1), there is removal of the outer coat with release of the single strand of RNA (2). The double-stranded RNA formed as a result of replication (3) now performs as interferon inducer through repressor-binding and derepression of the interferon gene in the host DNA, followed by transcription (4) and translation of the interferon (IF) messenger RNA by the cellular ribosomal system (5). New, mature viral particles may also be produced as shown (6, 7, 8). (II) The interferon proteins are rapidly released and may enter new cells, or perhaps act at the cell surface, to derepress the antiviral polypeptide gene in the host DNA. This is followed by transcription (9) to antiviral (AV) messenger RNA and translation (10) to the antiviral polypeptide. Viral replication is thus prevented, probably by inhibition at the ribosomal level (11).

on exhibits an antiviral effect early during the first 3 to 4 days in a viral infection, after which the host cells appear refractory to its action for a short time. The antiviral effect does not result from the direct inactivation of the virus or from nonspecific toxic effects on the cells. Rather it reacts with cells to induce the formation of a new intracellular substance, a polypeptide or small protein. In some manner, interferon protects susceptible host cells from translating viral-coded messages, yet does not interfere with the cell's translation of messages into host cell proteins. In Figure 1 is presented a brief diagrammatic synopsis of recent concepts in the production and mechanism of action of interferon.

Tab. 1. Serum interferon titres of RLV-inoculated and uninoculated control 3-month-old mice of different strains.

Strain	RLV-inoculated	Control
NZB	90	<16
BALB/c	330	<16
C57Bl/6	1024	24

(From Siegel et al.[4]. By permission of Journal of Immunology).

We first carried out comparative studies[4] on interferon induction by Rauscher virus, a murine leukemogenic virus (RLV), in three inbred strains of mice - NZB, BALB/c and C57Bl/6. Mice of the same age and strains served as uninoculated controls (Tab. 1). Interferon assays at 26 hours post inoculation demonstrated that interferon levels were lower for the NZB than for the BALB/c mice and highest for C57Bl/6. Interestingly, the BALB/c evinced less leukemia development than the NZB (Fig. 2), while the C57Bl/6 were typically refractory to RLV infection. Parenthetically, both NZB and BALB/c possess the

H-2^d histocompatibility allele while C57Bl/6 is H-2^b. However, De Maeyer et al.[5] have found no correlation between the H-2 allele type and high or low interferon production.

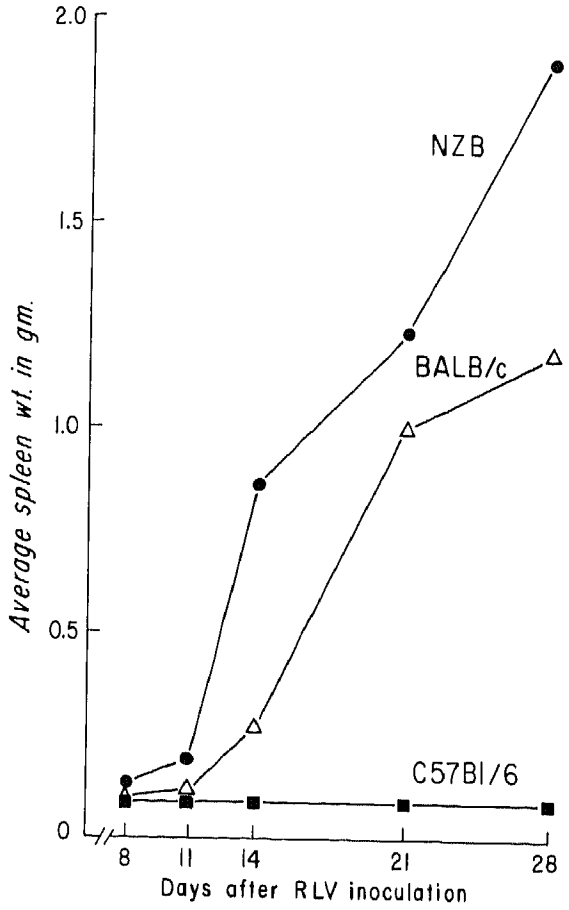

Fig. 2. Spleen weight changes for NZB, BALB/c and C57Bl/6 mice inoculated intravenously with RLV (0.20 ml of a 0.1% spleen cell extract) at 42 days of age. Each point represents the average of 4 - 6 mice.

With regard to the possible origin of the circulating interferons induced in mice by RLV, x-irradiation experiments would seem to implicate the hemopoietic system[6]. Two age groups of

the NZB strain, 6 and 13 months old, were exposed to 925 and 503R whole body x-irradiation respectively. After 4 days, five irradiated and five control mice in each group were inoculated with 0.2 ml of a 40% Rauscher virus suspension and bled 30 hours later for interferon assay. The data (Tab. 2) demonstrate that RLV-induced interferon production was highly radiosensitive, which would suggest that the radiosensitive lymphocyte might be involved in this production.

Tab. 2. Serum interferon titres in x-irradiated and control NZB mice inoculated with RLV.

Age (months)	Radiation Dose (R)	Interferon titres	
		Control	x-ray
6	925	100	<16
13	503	315	<16

(From Siegel et al.[4]. By permission of Journal of Immunology).

It might be mentioned that reports of observations in some murine systems have indicated inconsistencies between host interferon levels and resistance to viral infection. For example, in a non-oncogenic murine viral system, a negative correlation was noted by Glasgow between interferon production and host resistance to encephalomyocarditis virus, whereas relatively high serum levels of interferon in RLV-infected mice was accompanied by resistance to the disease. Also, Vandeputte et al.[7] were unable to prevent the splenomegalic response in mice to RLV by interferon administration, whereas Gresser et al.[8] found that repeated inoculation of interferon was effective in inhibiting development of splenomegaly.

In one of our early experiments[9], we were able to demonstrate

an enhancing effect of a vitamin C regimen on interferon induction by the Rauscher virus. BALB/c male mice were fed L-ascorbate (250 mg%) in their drinking water ad libitum, beginning at 24 days of age, for 3 months; a similar set of control animals remained on untreated water. At this time mice were stimulated to interferon production with the Rauscher virus. Twenty-seven hours after RLV inoculation, animals were bled for interferon assay by a colorimetric method[10] for quantifying cytopathic effects in mouse L-cell monolayers with vesicular stomatitis virus (VSV) as the challenge virus. Individual blood samples were collected by orbital sinus puncture and allowed to clot, and the serum was removed.

One-ml volumes of serial dilutions of sera were used for assay, and interferon titres were expressed as the reciprocal of the dilution which gave an absorbance reading at 540 nm midway between that of uninfected cell controls (100%) and the virus-infected controls (0%). Assays of sera were carried out twice with two different dosages of challenge virus, namely, 16×10^4 and 8×10^4 plaque-forming units (PFU) of VSV. A standard reference interferon preparation (National Institutes of Health Mouse Reference Interferon G002-904-511) was included with each assay. The viral inhibitor was partially labile at pH 2.0, not sedimentable at 100,000 x g, nondialyzable, inactivated by trypsin, nontoxic to L cells, and did not directly inactivate VSV - properties which, collectively, characterize the inhibitor as interferon.

BALB/c mice on the ascorbate regimen showed on an average (Tab. 3) a 62% increase in circulating interferon level when the assay was performed with a virus dose challenge of 16×10^4 PFU and a 145% increase at the lower dose virus challenge of 8×10^4 PFU, or an average increase of 104% based on the assays. Since the primary host defense in virus disease is probably the pro-

duction of interferon, the enhanced interferon response noted here in mice on the ascorbate regimen might suggest a mechanism to account, at least in part, for instances where vitamin C may provide protective effects against viral infection. It is of additional interest that the interferon-stimulating agent employed here was a leukemogenic virus (RLV), and that Gresser et al.[8] were able to inhibit development of the splenomegalic response in mice to this virus by interferon administration.

Tab. 3. Assays of serum interferon carried out in mouse L-cell monolayers at two different dosages of vesicular stomatitis challenge virus.

Mouse Group	Treatment	Interferon titres[a] 16×10^4 PFU	Interferon titres[a] 8×10^4 PFU
1	Ascorbate in water	480 (515)[b]	1,550 (1,668)
2	Ascorbate in water	550	1,785
3	Control	295 (318)	590 (682)
4	Control	340	775
	NIH reference	4,710	16,400

[a]Titres are expressed in dye-uptake units (DU_{50}/ml).
[b]Figures in parentheses are the average interferon titres for the two groups in a given treatment.
(From Siegel[9]. By permission of Infection and Immunity).

In this context, the protective effect of vitamin C in Rauscher viral leukemogenesis was next investigated. Rauscher virus produces an early erythroblastosis; large numbers of immature cells of the erythroid series are observed in the peripheral blood and also in the red pulp of the spleen and in the sinusoids of the liver[11]. Figure 3 is a peripheral blood smear showing these red blood cell precursors along with abnormal-

appearing mature erythrocytes showing varied sizes and shapes. Seen also are so-called smudge cells, disintegrated cells that could not be identified, and in which only the nuclear chromatin is recognizable. The erythroblastosis is accompanied by anemia and marked hepatosplenomegaly with deterioration of the general condition and death of the animal.

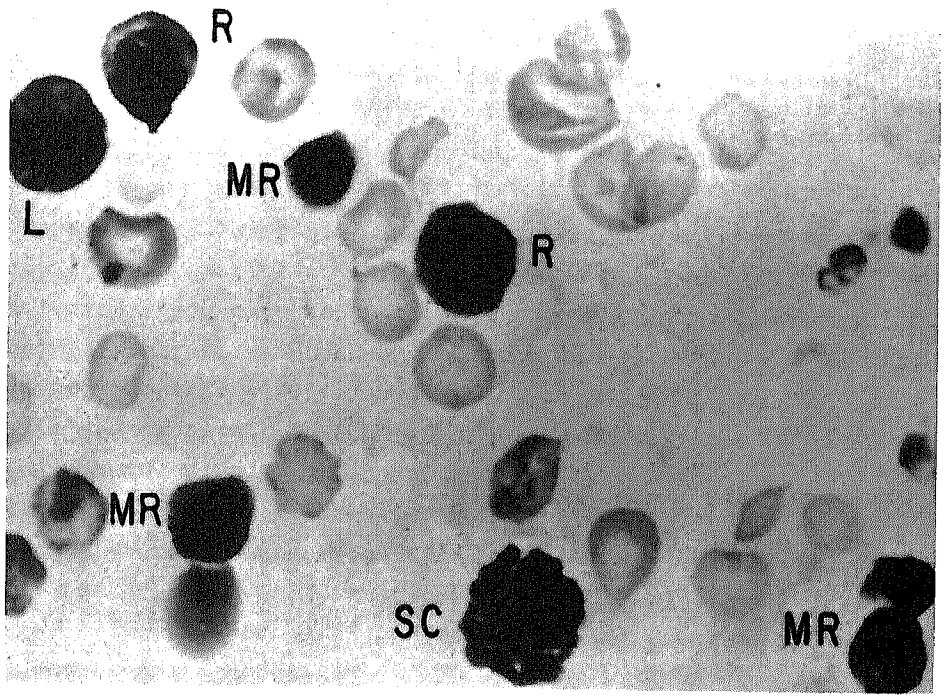

Fig. 3. Mouse peripheral blood during Rauscher leukemogenic virus infection. Legend: R (rubricyte), MR (metarubricyte), L (lymphocyte), SC (smudge cell). Also seen are abnormal-appearing mature erythrocytes showing anisocytosis, poikilocytosis and polychromatophilia. Wrights-Giemsa x 1400. (From Siegel and Morton, In Conference on Immunobiology of Cancer, Nov. 1975. By permission of Annals of New York Academy of Sciences.)

After 2 weeks on a regimen of 250 mg% vitamin C in the drinking water, DBA/2 strain mice were inoculated intraperitoneally with

a 10% or 1% spleen cell extract of RLV, and the vitamin C regimen continued to termination of the experiment. Splenomegaly is seen (Tab. 4) to be considerably diminished in the animals on vitamin C. However, it should be noted that at the high dosages of virus employed, some disease development (spleen weight >0.25 g) was evident in most infected mice. In this connection, present studies in progress involve less concentrated viral inocula, to determine if the disease can be completely prevented, or its onset significantly delayed.

Tab. 4. Protective effect of vitamin C on Rauscher viral leukemogenesis[a].

RLV dose	No. Mice	Treatment	Spleen weights (mg)	Significance
10^{-1}	24	Vitamin C	512.1 ± 29.0[b]	p <0.001
	21	Control	818.7 ± 41.9	
10^{-2}	15	Vitamin C	395.2 ± 44.0	p <0.025
	12	Control	733.2 ± 90.1	

[a]DBA/2J female mice were started on a regimen of 250 mg% vitamin C in the drinking water at 7 weeks of age. They were inoculated with RLV (10^{-1} or 10^{-2} dilution of infected spleen extract) two weeks later. The vitamin C regimen was continued to termination of the experiment, 3 weeks in the case of 10^{-1} RLV and 4 weeks for 10^{-2} RLV.
[b]Mean ± S.E.M.

We then went on to a study[12] of this phenomenon in vitro employing cultures of mouse cells - transformed L cells and normal embryonic fibroblasts - stimulated by the synthetic polynucleotide polyriboinosinic-polyribocytidylic acid (poly(rI) · poly(rC)), a double-stranded RNA which induces interferon. Here we observed that the addition of L-ascorbate to cultures of mouse cells resulted in increased synthesis of interferon.

The presence of ascorbate in cultures of L cells resulted in increased interferon production as demonstrated in three separate experiments. Values for 10^{-5} M and 10^{-4} M ascorbate (Tab. 5) were seen to show a dose response effect. Control cultures (no ascorbate added) produced a mean interferon titre of 1,405 ± 285. All three observations made with 10^{-4} M ascorbate exceeded this control value by more than three standard deviations and hence are considered significant.

Tab. 5. Effect of ascorbate on interferon induction by poly(rI) • poly(rC) in mouse L-cell cultures.

Concentration of ascorbate (M)	Interferon titres[a]		
	Experiment 1	Experiment 2	Experiment 3
None added	1,450	1,100	1,665
1.0×10^{-5}	1,785	ND	1,910
1.0×10^{-4}	3,330	3,105	5,060
NIH reference	20,185	15,300	28,545

[a]Titres are expressed in DU_{50}/ml.
ND, not done.
(From Siegel[12]. By permission of Nature).

It is well known that cells maintained in culture lose their resemblance to normal cells. In this connection, we have observed that primary and secondary cultures of mouse embryo fibroblasts are considerably less responsive to interferon induction by poly(rI) • poly(rC) than are cells of the mouse L line. Therefore, it seemed important to study the effects of ascorbate on cells newly established in culture. The results of two experiments (Tab. 6) with secondary cultures of normal mouse embryo fibroblasts revealed that, as in the case of the L cell line, 10^{-4} M ascorbate substantially increased the production of interferon. Statistically, the interferon

titres observed with 10^{-4} M ascorbate in both cases exceeded the mean value of 127.5 ± 46 for control titres by more than three standard deviations.

Tab. 6. Effect of ascorbate on interferon induction by poly(rI) · poly(rC) in normal mouse embryo fibroblast cultures.

Concentration of ascorbate (M)	Interferon titres[a]	
	Experiment 1	Experiment 2
None added	95	160
1.0×10^{-4}	310	415
NIH reference	11,600	26,635

[a]Titres are expressed in DU_{50}/ml.
(From Siegel[12]. By permission of Nature).

The role of ascorbate in potentiating the production of interferon is not clear. Vitamin C may affect the interactions of poly(rI) · poly(rC) at the cell surface or within the cell. Conceivably, ascorbate could function to increase the amount of interferon messenger RNA available for translation in cells stimulated with the polynucleotide by inhibition of a regulatory protein[13] and promotion of the stability of the interferon messenger RNA[14]. There have been reports of stimulation of the antiviral activity of interferon by cyclic AMP[15] and, more recently, by cyclic AMP and certain of its synthetic derivatives[16]. In this connection, preliminary experiments have not been suggestive of significant differences in cyclic AMP levels between untreated and ascorbate-treated L cells as determined by radioimmunoassay. However, it has been reported[17] that ascorbic acid inactivates phosphodiesterase (PDE) and therefore should result in increased concentrations of cyclic AMP. In any case, the mechanism of ascorbate in enhancing interferon syn-

thesis in mouse cells in vitro, as well as in the intact animal, remains to be elucidated.

In the intact animal, fixed and circulating cells of the reticuloendothelial system appear to be good producers of interferon, although high interferon titres are often found at the tissue site of greatest viral replication. While viruses are capable of stimulating the production of interferon in virtually all nucleated cell types, in animals injected with a number of viruses lymphocytes appear to be responsible for the production of circulating interferon. Antilymphocyte serum, for example, has been observed to destroy the capacity of human leukocyte suspensions incubated with myxoviruses to produce interferon. Also, a parallel diminution has been noted[18] in peripheral lymphocyte numbers and serum interferon titres in mice exposed to x-irradiation. Because of its demonstrated interferon-enhancing effect, and because of the apparent dual function of lymphocytes in antibody production and interferon formation, experiments were undertaken to determine whether ascorbate might also play a role in influencing the immune response.

The humoral immune response of ascorbate-treated mice against sheep red blood cells (SRBC) was first investigated. In the plaque assay[19] employed for this purpose, spleen cell suspensions from mice immunized with sheep red blood cells are incorporated into a supporting medium containing SRBC and guinea pig serum complement. Zones of hemolysis develop around each antibody-producing cell, and the numbers of such cells are then counted. The plasma cell is the lymphocyte of the immune system that makes antibody against the sheep cells. Antibodies secreted by the plasma cell, referred to as the plaque-forming cell (PFC), destroy the red blood cells in the area into which the antibodies have diffused producing a zone of hemolysis or a plaque. Here (Tab. 7), little effect was noted on spleen anti-

Tab. 7. Effect of vitamin C on antibody response to SRBC in BALB/c mice[a].

Experiment	Treatment	Days on vitamin C before immunization	PFC/spleen x 10^{-3} mean ± SEM
I	Vitamin C	9	163.7 ± 4.359
	Control		146.2 ± 17.21
II	Vitamin C	26	175.1 ± 55.90
	Control		160.7 ± 38.40
III	Vitamin C	26	59.6 ± 3.44
	Control		64.8 ± 4.37
IV	Vitamin C	35	328.2 ± 37.2
	Control		284.1 ± 6.33
V	Vitamin C	67	86.2 ± 7.93
	Control		73.6 ± 6.09

[a]Mice were placed on a vitamin C regimen (250 mg%) at 3.5 - 4.5 months of age. After the indicated number of days, animals were injected i.p. with 0.20 ml of a 1 : 10 suspension of washed sheep red blood cells (SRBC) and vitamin C treatment continued. Direct spleen plaque forming cells were assayed on days 4 or 5. Each value represents the mean for 3 - 5 mice. Differences between vitamin C and control mice were not significant ($p > 0.05$).

body plaque formation in BALB/c mice after drinking vitamin C for periods of 9 to 67 days before immunization.

Sheep erythrocyte antigens are thymus-dependent, that is, for the induction of antibody response a collaboration between thymus-derived lymphocyte (T cell) and bone marrow-derived lymphocyte (B cell) is necessary, the former providing a "helper" function to the antibody precursor B cell. In contradistinction, the response to lipopolysaccharide (LPS) antigen is T-lymphocyte-independent. The data in Table 8 indicate that the antibody response to LPS, uniquely a B-lymphocyte response, was not significantly different in the vitamin C and control animals.

Tab. 8. Effect of vitamin C on the response of BALB/c mice to immunization with E. coli lipopolysaccharide[a].

Experiment	Treatment	Days on vitamin C before immunization	PFC/spleen x 10^{-3} mean ± SEM
I	Vitamin C	9	11.9 ± 1.69
	Control		15.6 ± 1.10
II	Vitamin C	15	15.0 ± 1.34
	Control		21.8 ± 2.00
III	Vitamin C	17	23.3 ± 2.20
	Control		27.4 ± 4.08
IV	Vitamin C	28	32.3 ± 8.01
	Control		28.0 ± 9.42
V	Vitamin C	28	22.7 ± 5.05
	Control		41.8 ± 10.5
VI	Vitamin C	29	21.1 ± 2.46
	Control		26.8 ± 4.06
VII	Vitamin C	29	25.9 ± 5.85
	Control		24.7 ± 3.02

[a]Mice were placed on a vitamin C regimen (250 mg%) at 2.5 - 3.5 months of age. After the indicated number of days, animals were injected intravenously with 5 µg E. coli lipopolysaccharide and vitamin C treatment continued. Direct spleen plaque-forming cells were assayed on days 4 or 5 using LPS-coated SRBC as substrate. Each value represents the mean for 3 - 5 mice. Differences between vitamin C and control mice were not significant ($p > 0.05$).

Endogenous colony forming cell (CFU) assays were performed by exposing mice to whole body x-irradiation (Picker-Vanguard, 71R/min at 277 kvp and 15 ma with a half-value layer of 1.9mm Cu). Nine days later, spleens were harvested and the surface colonies counted. Endogenous colony formation, a measure of the hemopoietic stem cell population in active growth cycle, has been noted with a number of strains of mice to parallel the extent of humoral antibody production[20]. In this connection, enumeration of the colony forming units (Tab. 9) in the spleens of mice irradiated at 400, 500 or 600 R showed no significant

differences between vitamin C-treated and control mice, in accord with the observed antibody responses (Tab. 7 and 8).

Tab. 9. Endogenous spleen colony formation (CFU) in BALB/c mice treated with vitamin C[a].

X-ray dose (R)	Vitamin C		Control	
	Spleen wt (mg)	CFU/spleen	Spleen wt (mg)	CFU/spleen
400	60.5 ± 2.46	22.4 ± 5.26	64.3 ± 4.15	18.2 ± 3.63
500	36.5 ± 2.41	2.4 ± 0.59	43.2 ± 1.30	5.2 ± 1.76
600	35.1 ± 2.72	0.50 ± 0.22	30.1 ± 1.40	0.20 ± 0.17

[a]Mice were started on a regimen of 250 mg% vitamin C in the drinking water at 2.5 months of age. Four weeks later animals were exposed to the designated x-ray dose and spleen colony formation and spleen weights measured 9 days later. Differences were not significant between vitamin C and control mice ($p > 0.05$).

The mitogen, concanavalin A (con A), stimulates T lymphocytes selectively and may be used as a sensitive probe to detect mouse splenic T cells which participate in cell-mediated immunity. In such experiments in which spleen lymphoid cells were stimulated in vitro with con A (Tab. 10), cells harvested from animals on a vitamin C regimen for 2 to 8 weeks showed significantly greater thymidine incorporation than controls, indicative of enhanced T-cell activity with vitamin C administration.

By way of concluding comments, the enhancement of interferon synthesis, and the augmentation of T-lymphocyte activity which is involved in the cellular immune response, would suggest the possibility of a multifactorial role for vitamin C in its suggested protective participation against viral infection. Vitamin C may stimulate T lymphocytes to proliferate or enhance their functional activity. As a consequence, an increase in

Tab. 10. Effect of vitamin C on response of spleen lymphoid cells to con A[a].

Experiment	Treatment	cpm Unstimulated	cpm con A	Δ cpm	Δ cpm mean ± SEM	Significance
			2 weeks			
I	Vitamin C	827 ± 49	12,866 ± 264	12,039	11,693 ± 283	
		609 ± 42	11,955 ± 127	11,346		
	Control	121 ± 5.5	1,447 ± 52	1,326		p <0.02
		426 ± 17	5,778 ± 178	5,352	3,404 ± 1,163	
		340 ± 8.5	3.873 ± 340	3,533		
			4 weeks			
II	Vitamin C	399 ± 6.4	41,353 ± 731	40,954		
		1,250 ± 31	53,984 ± 521	52,734	42,490 ± 4,784	
		682 ± 40	34,465 ± 712	33,783		
	Control	613 ± 18	30,277 ± 342	29,664		p <0.05
		341 ± 25	21,460 ± 335	21,119	21,068 ± 4,311	
		436 ± 27	12,856 ± 342	12,420		
			8 weeks			
III	Vitamin C	1,427 ± 8.5	56,724 ± 1,544	55,297		
		879 ± 29	57,079 ± 399	56,200	47,666 ± 7,003	
		456 ± 20	31,957 ± 261	31,501		
	Control	387 ± 16	6,179 ± 198	5,792		p <0.05
		485 ± 48	8,996 ± 90	8,511	12,976 ± 5,089	
		573 ± 21	25,197 ± 45	24,624		

[a]BALB/c mice were placed on a vitamin C regimen (250 mg% in drinking water) at 2.5 months of age. At 2 weeks (males) and at 4 and 8 weeks (females) spleen lymphoid cells were harvested for con A stimulation. Values presented are of assays for individual mice carried out in groups of 2 to 3 vitamin C and control animals. The different time points represent 3 separate experiments. Assays of ^{3}H-thymidine incorporation were performed in triplicate in each case. Mitogenic response studies were performed essentially as described previously[21]. The mitogenic response for each individual experiment is expressed as the difference in radioactivities (Δ cpm) between con A stimulated and unstimulated cultures.

the number of specific antigen-reactive cells could occur. The subsequent interaction of these T cells with viral antigen releases a variety of soluble effector molecules, or lymphokines, which are probably responsible for effecting cell-me-

diated immune responses. Among these are a factor which inhibits macrophage migration and an activation factor which stimulates macrophages to heightened microbicidal activity, thus resulting in more ready ingestion and degradation of infectious agents. In this way, the virus infection may be contained and possibly eventually eradicated from the host. Conceivably, this protective role of vitamin C could be expanded to include some forms of neoplasia, at least as intimated by our earlier preliminary findings with a virus-induced leukemia in mice (Tab. 4).

In point of fact, there is some evidence now that interferon may itself be a factor responsible for the activation of macrophages to a cytotoxic state, and thus be implicated in destruction of tumor cells (Fig. 4). Huang et al.[21] have shown that macrophages recovered in the peritoneal exudate from normal mice become activated when incubated with interferon. These authors have suggested that interferon may be the factor responsible for this activation and non-specific cytotoxicity for a variety of tumor cells. Also, normal macrophages can become activated by exposure to dsRNA and endotoxin[22], both

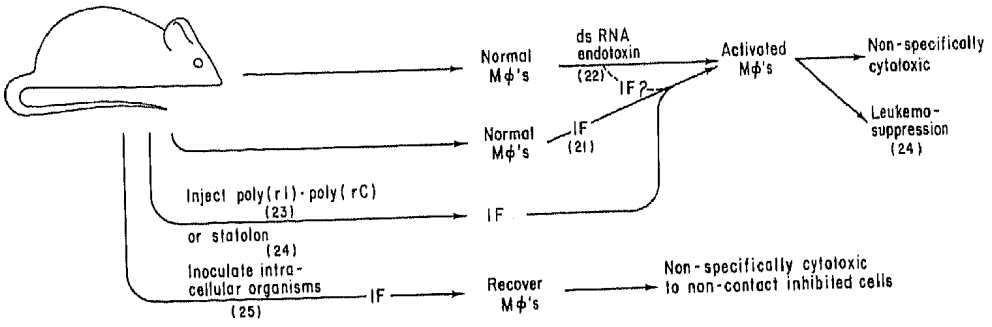

Fig. 4. Pathways of interferon activation of macrophages to in vitro cytotoxicity for tumor cells. Abbreviations used are: M φ's, macrophages; dsRNA, double-stranded RNA; IF, interferon. Numbers in parentheses are references to the literature.

of which are good interferon inducers, and are then similarly non-specifically cytotoxic. Polyriboinosinic acid · polyribocytidylic acid has been demonstrated to slow the growth of transplanted tumors[23], which may reflect activation of macrophages in the tumors by the induced interferon. Inoculation of statolon, an inducer of interferon production, into Friend leukemia virus-infected mice was found[24] to establish a dormant infection. Finally, peritoneal macrophages recovered from mice infected with an intracellular organism, such as Toxoplasma gondii, a good interferon inducer, are non-specifically and selectively cytotoxic for non-contact-inhibited cells[25]. A macrophage population selectively killing noeplastic cells may have considerable importance for surveillance, since such a cell population might eliminate neoplastic cells as they arise in vivo[26].

The studies described in this paper indicate that vitamin C enhances interferon formation and also plays a role in modulating the immune response. While the inclusion of vitamin C in the drinking water was without effect on the humoral antibody response, there was significantly increased cell-mediated immune reactivity as demonstrated by increased T-lymphocyte responses to concanavalin A. Figure 5 represents a delineation of the role of vitamin C in its suggested protective participation against viral disease, in this case against respiratory viral infection.

The search for antiviral agents is being actively pursued by the pharmaceutical companies and other research establishments. One of these, interferon, interferes with the synthesis of a wide range of viruses, is nontoxic and nonallergenic, and shows a high degree of species-specificity in relation to the cells on which it is active. It is, thus, potentially one of the most useful of all viral inhibitors, since it is completely

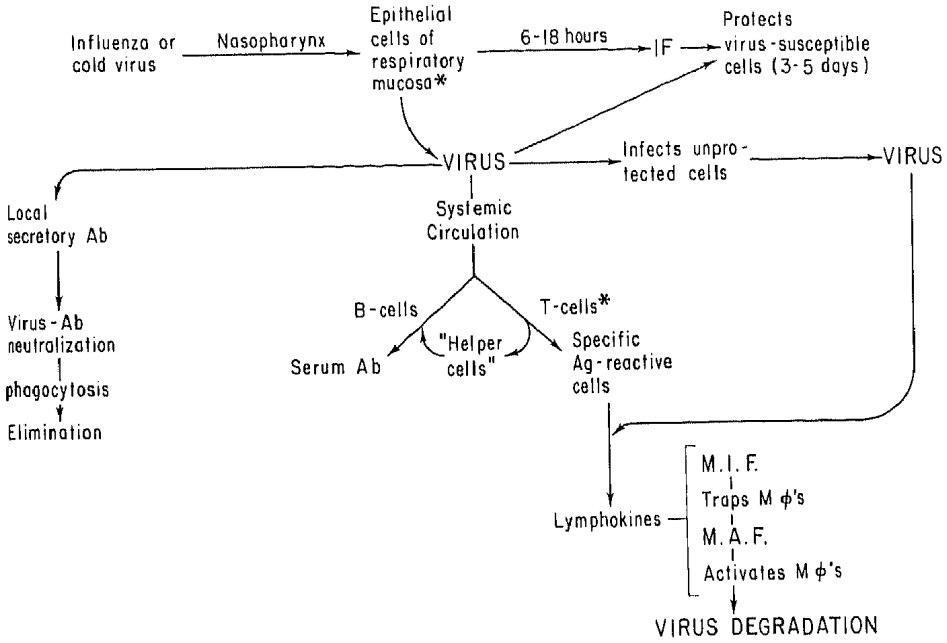

Fig. 5. Flowchart of respiratory virus-host cell interactions which effect host defense responses. Abbreviations used are: IF, interferon; Ag, antigen; Ab, antibody; M ϕ's, macrophage, M.I.F., migration inhibitory factor; M.A.F., macrophage activating factor.
*Vitamin C probably acts principally at these two points resulting in increased interferon synthesis by epithelial cells of the respiratory mucosa, which would provide increased protection against virus replication in susceptible cells; and in enhanced T-lymphocyte responsiveness, which would eventuate in specific antigen-reactive T cells with augmented production of lymphokines subsequent to interaction of virus with sensitized cells. The protective mechanism provided by vitamin C may thus be of a multifactorial nature, implicating the collaboration of both immunologic and non-immunologic processes.

nontoxic and can be used against a broad spectrum of viruses. Although interferon is harmless, and in certain instances proven effective, administration of exogenously produced interferon has not been promising in terms of prophylaxis or therapy, mainly due to the limiting factors associated with production and cost.

There appears at present to be little chance of producing and purifying the very extensive quantities of human interferon that would be required for practical use. A more feasible approach would involve the administration of interferon inducers. Double-stranded synthetic polynucleotides, especially polyriboinosinic · polyribocytidylic acid, have proved to be very effective inducers of interferon. Poly(rI) · poly(rC) has been used with success in the treatment of a number of viral infections in experimental animals and humans. However, clinical trials have demonstrated it to be highly toxic for humans, when administered systemically. The possibility remains that other nontoxic, interferon-inducing synthetic polynucleotides may become available for clinical use. Alternatively, vitamin C, because of its property of interferon enhancement, may conceivably prove useful as an adjuvant to the interferon-inducing effects of low, nontoxic levels of polynucleotides such as poly(rI) · poly(rC).

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