kinetic and other studies to establish that the activity is attributable to perfectly normal myophosphorylase enzyme. Nevertheless, these observations suggest that genetic coding for the development of a form of myophosphorylase activity is present in precursor cells of the regenerating skeletal muscle fibers of patients with myophosphorylase deficiency, and is expressed as enzymatic activity in regenerating fibers both in vitro and in vivo. Several hypotheses may be considered to explain the subsequent absence of phosphorylase activity (*11*, *12*) in mature fibers in vivo. (i) With muscle fiber maturity there may be loss of a specific enzyme which normally maintains phosphorylase production, survival, or activity. (ii) An abnormal specific protease may develop in the mature fibers which selectively inactivates myophosphorylase. (iii) A normally repressed "myophosphorylase repressor gene" may be derepressed, inhibiting the gene for myophosphorylase production. (iv) A normally present but inactive myophosphorylase inhibiting or destroying enzyme (or other factor) may become active, impairing myophosphorylase enzyme survival or activity.

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References and Notes

1. B. McArdle, *Clin. Sci.* **10**, 13 (1951).
2. R. Schmid and R. Mahler, *J. Clin. Invest.* **38**, 2044 (1959); C. M. Pearson, D. G. Rimer, W. F. Mommaerts, *Clin. Res.* **7**, 278 (1959).
3. P. W. Robbins, *Fed. Proc.* **19**, 193 (1960); L. P. Rowland, S. Fahn, D. L. Schotland, *Arch. Neurol.* **9**, 325 (1963).
4. M. B. Bornstein, *Lab. Invest.* **7**, 134 (1958).
5. Cultures were prepared as follows: Collagen-coated cover slips were conditioned for 24 hours with one drop of nutrient media (54 percent MEM with Hanks salts; 34 percent human placental cord serum; 10 percent mouse embryo extract; 600 mg of glucose per 100 ml; Pen-Strep (50 units of penicillin per milliliter of nutrient and 50 μg of streptomycin per milliliter of nutrient). A spinal cord explant from a 12- to 15-day in utero mouse embryo was added to each cover slip, fed one drop of media, and sealed into the Maximow chamber. After growing for 3 to 4 days, one to three explants of biopsied human muscle were added to the cover slip that contained spinal cord, with the muscle separated from the spinal cord by 4 to 8 mm. This degree of physical separation of spinal cord and muscle explants excluded functional innervation. The cultures were then fed three times a week.
6. All three patients met these four diagnostic criteria: (i) A history of muscle pain and cramps provoked by exercise, often limiting exercise, cases 1 and 2 having had pigmenturia following strenuous exercise; (ii) absence of a venous lactate rise with ischemic exercise, which produced contractures in cases 1 and 3; (iii) absence of striated muscle phosphorylase activity histochemically, with phosphorylase activity being present in the smooth muscle of blood vessels; and (iv) apparently excessive muscle glycogen histochemically. In addition, patients 2 and 3 were brother and sister; biochemical assay of the first muscle biopsy from the brother showed complete absence of total phosphorylase activity.
7. T. Takeuchi and H. Kuriaki, *J. Histochem. Cytochem.* **3**, 153 (1955).
8. M. T. Porter, A. L. Fluharty, H. Kihara, *Proc. Nat. Acad. Sci. U.S.A.* **62**, 887 (1969); *Science* **172**, 1263 (1971).
9. M. Vaughan, *J. Biol. Chem.* **235**, 3049 (1960).
10. Histochemistry of the fresh frozen muscle biopsy specimens was performed by Guy G. Cunningham.
11. O. A. Walsh, E. G. Krebs, E. M. Reimann, M. A. Brostrom, J. D. Corbin, J. P. Hickenbottom, T. R. Soderling, J. P. Perkins, *Advan. Biochem. Psychopharmacol.* **3**, 265 (1970).
12. E. H. Fischer, S. S. Hurd, P. Koh, V. L. Seery, D. C. Teller, *Control of Glycogen Metabolism* (Universite Tsforlaget, Oslo, 1968), pp. 19–33.

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Mouse Leukemia: Depression of Serum Interferon Production

Abstract. *Production of circulating interferon is significantly impaired in AKR/J mice after development of lymphoblastic leukemia and in Balb/c mice with clinical signs of Friend erythroblastic leukemia. This alteration has been observed with three interferon inducers, each one known to elicit an interferon response in different cells.*

Induction of interferon in vivo is currently being explored as a possible approach to the therapy of viral diseases and also of certain forms of neoplasia (*1*). I therefore report the marked inhibition of the production of interferon observed both in Balb/c mice with Friend virus–induced erythroblastic leukemia and in AKR/J mice after development of lymphoblastic leukemia.

Friend leukemia was induced in 6- to 8-week-old Balb/b mice by intravenous injection of a 1 to 10 (weight to volume) spleen extract prepared from the fourth passage in Balb/c mice of a Friend virus suspension, routinely maintained in Swiss mice (*2*). This virus suspension caused a palpable increase of spleen size in about 10 days, with an ensuing mortality of nearly 100 percent 8 to 10 weeks after inoculation. Production of circulating interferon was measured when the size of the spleen had increased significantly, that is, usually about 3 weeks after inoculation of Friend virus (Fig. 1). Three different interferon inducers were tested, each stimulating serum interferon production in a different cell system: (i) Newcastle disease virus (NDV), a paramyxovirus that induces interferon synthesis mainly in lymphocytes (*3*); (ii) encephalomyocarditis virus (EMC), a small RNA virus that stimulates interferon production in radioresistant cells that are not derived from bone marrow stem cells [(*4*) and unpublished results]; and (iii) polyriboinosinic-polyribocytidylic acid [poly(I) · poly(C)], a synthetic polyribonucleotide that induces interferon synthesis in a radioresistant cell population derived from hemopoietic stem cells, probably macrophages [(*5*) and unpublished results]. Origin of virus strains, titers, and mode of cultivation have been published (*6*). Poly(I) · poly(C) (P-L Biochemicals Inc., Milwaukee, Wisconsin) was resuspended in Field's phosphate buffer (*7*) at a concentration of 250 μg/ml before use. Figure 1 represents individual serum interferon levels, measured at the peak of production, in Balb/c mice with and without Friend leukemia. For all three interferon inducers, a highly significant ($P < .001$) decrease of interferon levels was observed in leukemic animals. The inhibition was related to the degree of splenomegaly, as shown in experiments with poly(I) · poly(C) and EMC, in which interferon response was measured at an early and a later stage of the disease. When the average spleen weight was about twice the normal value, that is, 250 mg, serum interferon levels were already significantly lower than those of control mice ($P < .01$); at a later stage of the disease, when spleen size averaged 2000 mg, serum interferon response had come down further, to represent only 5 to 10 percent of that obtained in healthy mice.

Similar results were obtained in the case of a spontaneous leukemia, the AKR mouse lymphoblastic leukemia. Results plotted in Fig. 2 show a highly significant ($P < .001$) decrease of production of serum interferon in leukemic AKR/J mice, 6 to 9 months old, injected intravenously with NDV or poly(I) · poly(C). In the control

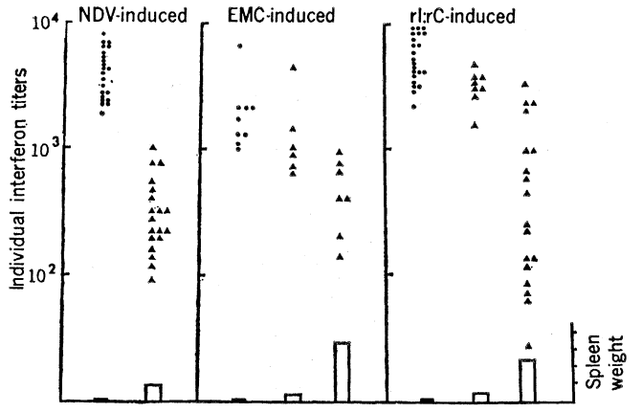

Fig. 1. Interferon production in Balb/c mice with Friend leukemia. Balb/c mice of both sexes, aged 6 to 8 weeks, were injected intravenously with a 1 to 10 (weight to volume) spleen extract, prepared from the fourth passage in Balb/c mice of a suspension of Friend virus, routinely maintained in Swiss mice (*2*). Production of circulating interferon was measured 3 weeks later. Mice were injected intravenously, by way of the orbital sinus, with 0.2 ml of the interferon inducer, at a dose corresponding to 10^8 egg infective doses, 50 percent effective, for NDV, 10^8 plaque-forming units in L cells for EMC or 50 μg for poly(I) · poly(C). Blood was drawn 9 hours after virus and 2 hours after poly(I) · poly(C). Interferon determinations were done in L cells, by using a plaque-reduction method, with VSV as challenge virus. Each value plotted in the figure represents the serum interferon titer, in units per 4 ml, of each individual serum. Symbols used are dots for control mice and triangles for Friend leukemic mice. Average spleen size in each group of mice is given in milligrams, each division on the scale corresponding to 500 mg. Abbreviation *rI : rC* is a shortened form of poly(I) · poly(C).

mice, NDV-induced serum interferon levels were about 30 percent lower in old preleukemic animals (6 to 9 months old) than in young ones (2 to 3 months old). A comparable effect of aging on the levels of interferon was reported earlier for mice of the Balb/c, C3H, and C57BL strains (*8*).

These experiments in Balb/c and AKR/J mice have revealed a drastic decrease of interferon-producing capacity in animals that have developed either an induced or a spontaneous form of leukemia. Moreover, the impairment of interferon production is correlated with the degree of leukemic involvement, as demonstrated in mice with Friend disease at two different stages of splenomegaly. Depression of circulating interferon response in mice infected with leukemia virus has been previously reported by other investigators. Thus Wheelock (*9*) and Wheelock and Larke (*10*) reported that Friend leukemia virus infection inhibited the interferon response to Sendai virus in DBA/2 mice 1 to 10 days after inoculation of the leukemia virus. However, 12 days after inoculation with Friend virus, Sendai virus was again able to induce high levels of interferon in the serum. Similarly, Vandeputte *et al.* (*11*) reported that Swiss albino and NMRI mice had a decreased serum interferon response to inoculation with Sindbis virus up to 14 days after injection of a suspension of Rauscher virus. The reduction in interferon production was first recorded 16 hours after inoculation with the virus and reached a maximum at 40 hours; from 64 hours to 8 days after inoculation of Rauscher virus, a progressive recovery of the interferon-producing capacity was observed, but without ever reaching normal levels again. The inhibitory effect of Friend and Rauscher leukemia virus on the in vivo interferon response, as reported by Wheelock and Vandeputte and their co-workers, must be clearly distinguished from the one presented here, because it occurs at an early stage of the infection with the leukemia virus and disappears progressively; in contrast, the inhibition of serum interferon response described in this report starts at a later stage of the leukemia and increases progressively with the advancement of the disease, until death of the animals.

The mechanism of inhibition of interferon production, as reported here, has not yet been defined. In vitro, human lymphoma cells may be poor producers of interferon as compared with normal white cells (*12*), and, in mice, replacement of normal leukocytes by leukemic cells could be partly responsible for the depressed levels of serum interferon. This possibility is supported by the fact that both NDV- and poly(I) · poly(C)–induced interferon originates in cells derived from hemopoietic stem cells. Keeping in mind that the bulk of EMC-induced interferon is made in cells that are not derived from the hemopoietic system, the hypothesis of a competition between normal and leukemic cells for uptake of inducer is less likely. The depression of interferon production observed in leukemic mice may be the result of the presence of circulating inhibitors of interferon production (*13*), and

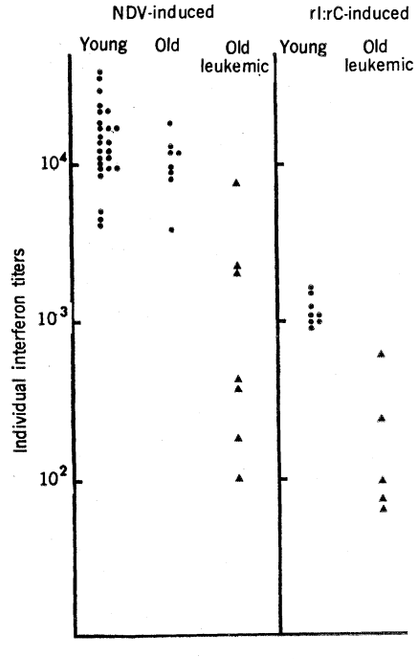

Fig. 2. Interferon production in AKR/J mice. AKR/J mice were injected intravenously with 0.2 ml of the interferon inducer, namely, 10^8 egg infective doses, 50 percent effective, for NDV or 50 μg for poly(I) · poly(C). Serum was collected and titrated for interferon as stated in the legend of Fig. 1. Animals of two different ages were selected—6- to 8-week-old females (*Young*) and 7- to 9-month-old retired breeders (*Old* and *Old leukemic*). Among the latter, preleukemic animals had not yet displayed any sign of leukemia, while the leukemic ones showed advanced signs of the disease, namely, significant increase of thymus, enlargement of peripheral lymph nodes, and invasion of peripheral blood by lymphoblasts. Each value given in the figure represents the titer of serum interferon, in units per 4 ml, of each individual serum. Symbols used are dots for nonleukemic mice and triangles for leukemic mice. Abbreviation *rI : rC* is a shortened form of poly(I) · poly(C).

further work will be required to examine this hypothesis. There are several papers describing the presence of various antagonists of interferon action in malignant (*14*) and embryonic tissues (*15*); these antagonists are of either viral or cellular origin. Inhibitors of interferon production have been less well documented. Isaacs *et al.* (*16*) have described a "blocker" of interferon synthesis in certain crude preparations of chick interferon, and Kato and Eggers (*17*) have reported the presence of a mucopolysaccharide depressor in chick chorioallantoic fluid. Knowing that interferon synthesis depends on a subtle balance between the inducer and cellular repressors (*18*), one can envisage an excess of such repressors in leukemic cells. Another attractive hypothesis would be to ascribe the impairment of interferon synthesis to the presence of leukemia virus particles within the cells. To our knowledge, no study of the effect of particles of mouse leukemia viruses on production of interferon has yet been carried out at the cellular level. For a tumorigenic DNA virus, the Epstein-Barr virus (EBV), Swart and Young (*19*) reported the existence of an inverse relation betwen spontaneous interferon production and virus content in human cell lines derived from Burkitt's lymphoma. Their observation, however, cannot be taken as a general rule, since other workers (*20*) could not find any correlation between interferon production and infection of the cultures by EBV, in various lymphoblastoid cell lines.

Whatever mechanism will turn out to be responsible for the depression of interferon synthesis in leukemic mice, the phenomenon, at this point, has a twofold implication: (i) together with the well-documented immunodepression found in leukemia (*21*), it may contribute to the lowered resistance to viral infections (*22*); and (ii) it could be an obstacle to the efficacy of interferon inducers in therapeutic trials of human leukemias, should an inhibition of interferon production also occur in man. That this may well be the case is suggested by the work of Armstrong *et al.* (*23*), who found significantly less interferon in vesicle-fluid during herpes zoster infection, in three out of seven patients with cancer, when compared with noncancerous patients.

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References and Notes

1. H. B. Levy, *Arch. Intern. Med.* **126**, 78 (1970).
2. C. Friend, *J. Exp. Med.* **105**, 307 (1957); P. Tambourin, F. Wendling, N. Barat, F. Zajdela, *Nouv. Rev. Fr. Hematol.* **9**, 461 (1969).
3. J. De Maeyer-Guignard and E. De Maeyer, *Nature New Biol.* **229**, 212 (1971).
4. E. De Maeyer, J. De Maeyer-Guignard, P. Jullien, *J. Gen. Physiol.* **56**, 43s (1970).
5. E. De Maeyer, R. M. Fauve, J. De Maeyer-Guignard, *Ann. Inst. Pasteur Paris* **120**, 438 (1971).
6. E. De Maeyer, J. De Maeyer-Guignard, P. Jullien, *Proc. Soc. Exp. Biol. Med.* **131**, 36 (1969).
7. A. K. Field, A. A. Tytell, G. P. Lampson, M. R. Hilleman, *Proc. Nat. Acad. Sci. U.S.A.* **58**, 1004 (1967).
8. E. De Maeyer and J. De Maeyer-Guignard, *J. Gen. Virol.* **2**, 445 (1968).
9. E. F. Wheelock, *Proc. Nat. Acad. Sci. U.S.A.* **55**, 774 (1966).
10. ——— and R. P. B. Larke, *Proc. Soc. Exp. Biol. Med.* **127**, 230 (1968).
11. M. Vandeputte, J. De Lafonteyne, A. Billiau, P. De Somer, *Arch. Gesamte Virusforsch.* **20**, 235 (1967).
12. S. H. S. Lee, R. L. Ozere, C. E. van Rooyen, *Proc. Soc. Exp. Biol. Med.* **122**, 32 (1966); S. H. S. Lee, C. E. van Rooyen, R. L. Ozere, *Cancer Res.* **29**, 645 (1969); H. Strander, K. Cantell, J. Leisti, E. Nikkila, *Clin. Exp. Immunol.* **6**, 263 (1970).
13. In experiments carried out in mice bearing transplantable lymphomas, E. De Maeyer and P. Jullien have observed a decreased serum interferon response to NDV, poly(I) · poly(C), and EMC. Moreover, they could demonstrate that the serum of these animals contained a factor capable of inhibiting interferon production in tissue culture (personal communication). R. B. Heath, from St. Bartholomew's Hospital (London), recently also observed a depressed interferon production in mice implanted with lymphoma (personal communication).
14. J. L. Truden, M. M. Sigel, L. S. Dietrich, *Virology* **33**, 95 (1967); C. Chany, J. Lemaître and A. Grégoire, *C. R. Acad. Sci. Ser. D* **269**, 2628 (1969); C. A. Brailowski, L. D. Berman, C. Chany, *Int. J. Cancer* **4**, 194 (1969).
15. F. Fournier, S. Rousset, C. Chany, *C. R. Acad. Sci. Ser. D* **266**, 2306 (1968); S. E. Grossberg and P. S. Morahan, *Science* **171**, 77 (1971).
16. A. Isaacs, Z. Rotem, K. H. Fantes, *Virology* **29**, 248 (1966).
17. N. Kato and H. J. Eggers, *J. Gen. Virol.* **5**, 369 (1969).
18. K. Paucker and M. Boxaca, *Bacteriol. Rev.* **31**, 145 (1967).
19. B. E. Swart and B. G. Young, *J. Nat. Cancer Inst.* **42**, 914 (1969).
20. B. A. Zajac, W. Henle, G. Henle, *Cancer Res.* **29**, 1467 (1969); A. T. Haase, J. S. Johnson, J. A. Kasel, S. Margolis, H. B. Levy, *Proc. Soc. Exp. Biol. Med.* **133**, 1076 (1970).
21. R. D. A. Peterson, R. Hendrickson, R. A. Good, *Proc. Soc. Biol. Med.* **114**, 517 (1963); M. H. Salaman and N. Weddeburn, *Immunology* **10**, 445 (1966); D. Metcalf and R. Moulds, *Int. J. Cancer* **2**, 53 (1967); W. S. Ceglowski and H. Friedman, *J. Nat. Cancer Inst.* **40**, 983 (1968); M. Bennett and R. A. Steeves, *ibid.* **44**, 1107 (1970).
22. See review by T. C. Merigan and D. A. Stevens, *Fed. Proc.* **30**, 1858 (1971).
23. R. W. Armstrong, M. J. Gurwith, D. Waddell, T. C. Merigan, *N. Engl. J. Med.* **283**, 1182 (1970).

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Neuroblastoma: Synchronization of Neurite Outgrowth in Cultures Grown on Collagen

Abstract. *Neurite outgrowth in cultured neuroblastoma cells is inhibited in cells grown on a collagen substratum as compared to those grown on glass. Rapid, synchronized initiation of neurite outgrowth occurs after hydrolysis of the underlying collagen with collagenase. Axonated cultures exhibit an increased RNA and protein content as compared to cultures grown on collagen.*

Suspension cultures of murine neuroblastoma appear as round, undifferentiated cells; when allowed to settle onto a glass substratum these cells extend multiple processes resembling neurites. Neurite formation (axonation), which may be considered a morphologic manifestation of differentiation in these cells, is associated with the appearance of specialized neural functions: electrical and chemical excitability of membranes, generation of action potentials, and production of neurotransmitter substances (*1*).

Several laboratories have reported procedures for enhancing or synchronizing axonation. Undifferentiated cells may be grown in suspension or in vessels with plastic surfaces to which they cannot adhere, and then transferred to vessels with glass or equivalent surfaces where neurite outgrowth ensues (*2*). Other approaches involve manipulation of culture medium constituents. Neurite outgrowth is suppressed in some clones grown adherent to glass in medium containing 10 percent serum, but rapid outgrowth of neurites occurs in most cells when such medium is replaced with one devoid of serum (*3*). Addition of certain drugs [5-bromodeoxyuridine (BrdU), 5-fluorodeoxyuridine (FdU), dibutyryl cyclic adenosine monophosphate (dibutyryl cyclic AMP), and prostaglandins E_1] to medium containing serum also stimulates axonation (*4*). We report here development of a new procedure for inducing rapid, synchronized axonation, which circumvents the use of culture transfers, nonphysiologic growth conditions, and toxic drugs.

We have found that neuroblastoma cells grown on a thin layer of native collagen are round and devoid of neurites, yet are firmly adherent to the collagen substratum. Addition of purified collagenase to the culture medium