Is a compromised interferon response an etiologic factor in Reye's syndrome?

K.R. ROZEE, PH D
S.H.S. LEE, PH D
J.F.S. CROCKER, MD
S. DIGOUT, M SC
E. ARCINUE,* MD

Young mice injected with sublethal doses of Toximul MP8, a typical commercial polyoxyethylene ether-based emulsifier, died more frequently when infected with encephalomyocarditis virus than did control mice. Lymphocytes taken from emulsifier-injected mice responded poorly to interferon induction, unlike lymphocytes from control animals. Interferon protected control mice against viral encephalomyocarditis, but such protection was not equally demonstrable in emulsifier-injected mice. These data suggest that the enhanced lethality of encephalomyocarditis virus in emulsifier-injected mice is associated with and perhaps caused by a compromised interferon response in these animals.

Since these emulsifiers are commonly found in the environment in areas where forests are sprayed with pesticides, a group of children suffering from Reye's syndrome who lived in such areas was investigated. Blood samples were obtained from five children with influenza B-associated Reye's syndrome during their acute illness and during convalescence. Lymphocytes obtained from these samples and from peripheral blood samples from healthy children (controls) were induced to synthesize interferon by exposure to Newcastle disease virus. The lymphocytes from the convalescent patients and from the controls responded well to induction. However, the lymphocytes obtained from patients during the acute phase of Reye's syndrome responded very poorly and produced significantly less interferon.

La mortalité chez des souris jeunes injectées avec des doses sublétales de Toximul MP8, un émulsifiant commercial typique à base d'éther de polyoxyéthylène, et infectées avec le virus de l'encéphalomyocardite a été plus grande que celle chez des souris témoins. Contrairement aux lymphocytes des animaux témoins, ceux prélevés de souris injectées à l'émulsifiant ont peu réagi à l'induction d'interféron. L'interféron à protégé les souris témoins contre l'encéphalomyocardite virale, mais cette protection n'a pu être également démontrée chez les souris ayant reçu l'émulsifiant. Ces résultats indiquent que l'augmentation de la létalité du virus de l'encéphalomyocardite chez les souris injectées à l'émulsifiant est reliée à et possiblement causée par une altération de la production d'interféron chez ces animaux.

Comme ces émulsifiants sont fréquemment retrouvés dans l'environnement dans les régions où les forêts sont arrosées avec des insecticides, on a étudié un groupe d'enfants vivant dans ces régions qui ont été atteints du syndrome de Reye. Des échantillons sanguins ont été prélevés chez cinq enfants souffrant d'un syndrome de Reye associé au virus influenza B durant la phase aiguë de la maladie et durant la convalescence. On a provoqué la synthèse d'interféron par les lymphocytes extraits de ces échantillons ainsi que d'échantillons de sang périphérique d'enfants sains (groupe témoin) en les exposant au virus de la maladie de Newcastle. Les lymphocytes des patients convalescents et ceux des témoins ont bien réagi à l'induction. Toutefois, les lymphocytes obtenus des patients durant la phase aiguë du syndrome de Reye ont très mal répondu et ont produit significativement moins d'interféron.

Reye's syndrome is a biphasic disease in which a damaging and frequently fatal encephalopathy and fatty degeneration of the viscera suddenly develop in children who had seemed to be recovering normally from influenza B or other virus infections.[1] Epidemiologic data show that the epidemic curve of this disease is coincident with that of influenza B[2-5] and strongly support the thesis that a factor present in rural environments is etiologically important.[2] In previous publications we presented data in support of our contention that one environmental factor that may be implicated is the use of commercial pesticide sprays in rural areas.[6-8] In Atlantic Canada the distribution of cases of Reye's syndrome has in previous years been found to coincide geographically with forested areas that have been sprayed every growing season since 1952 with pesticides to suppress the spruce budworm that infests the coniferous forests of that region. The populace of neighbouring unsprayed forests had, and still has, a significantly lower incidence of this syndrome than the populace of the sprayed area. The component of the sprays that we believe may be implicated in the increased incidence in sprayed areas belongs to a class of emulsifiers chemically based on polyoxyethylene polymers.[9] The mechanism by which these emulsifiers act to thwart the normal recovery of children from

From the departments of microbiology and pediatrics, Izaak Walton Killam Hospital for Children and Dalhousie University, Halifax and *the Detroit Children's Hospital, Detroit, Michigan

Reprint requests to: Dr. K.R. Rozee, Department of microbiology, Dalhousie University, Halifax, NS B3H 4H7

certain virus infections likely arises from their ability to compromise the interferon response. We have reported that these chemicals not only enhance virus penetration of host cells, but also prevent these cells from responding both to an interferon inducer and to interferon itself.[10,11]

We now report on the response of young mice that were exposed to Toximul MP8, a typical commercial polyoxyethylene ether-based emulsifier used widely as a dispersal agent in pesticide sprays, and then infected with encephalomyocarditis virus and on the induction of interferon in the peripheral lymphocytes of these mice by a normally successful induction method.

We also compare the results of interferon induction in lymphocytes taken from five children at the time they were suffering from influenza B-associated Reye's syndrome with the results in lymphocytes from the same children following their recovery and from control children.

Materials and methods

Cell cultures and viruses

Established lines of mouse L-929 cells and of monkey BSC-1 kidney cells were grown as monolayer cultures in minimum essential medium, Eagle-growth, supplemented with 10% heat-inactivated fetal bovine serum.

Cultures of stock bovine vesicular stomatitis virus, strain Indiana (used as a challenge virus in interferon assays), and of mouse encephalomyocarditis virus (used in animal studies) were prepared in L-929 cells and stored at –70°C.

Stock Newcastle disease virus, strain California was propagated in the allantoic cavity of 9-day-old embryonated chicken eggs. The infectivity of the virus was titrated to approximately 3×10^9 plaque-forming units by applying primary chick embryo fibroblasts under an agar overlay. The virus was then inactivated by ultraviolet light to prepare it for use as an interferon inducer. These methods and those for interferon plaque-reduction assays have been described previously.[12]

Mice

SWR male and female adult mice were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. All matings were carried out in our laboratory so that neonates of particular ages were available when required.

Determination of 50% lethal dose (LD_{50})

The lethality of Toximul MP8 (provided by Charles Tennant and Co., Ltd., Toronto) and of the encephalomyocarditis virus to mice was evaluated by recording the rate of survival of the mice 2 weeks after they had received one intraperitoneal injection of 0.1 ml of graded amounts of the test material in phosphate-buffered saline. The LD_{50} was determined by the method of Reed and Muench,[13] with 8 to 10 mice being used for each concentration in the dilution series, and was found to be about 170 mg of Toximul MP8 per kilogram of body weight per 0.1 ml of test solution.

Lymphocyte preparations

Heparinized human peripheral blood in 5-ml portions was obtained from healthy donors and from patients with Reye's syndrome. The blood was diluted 1:1 with Hanks', balanced salt solution (HBSS), layered onto 3.5 ml of Lymphoprep (Accurate Chemical and Scientific Corporation, Hicksville, New York), and centrifuged at room temperature for 30 minutes at $375 \times g$ in an International centrifuge (model PR2), as we described previously.[14] In brief, the lymphocyte-enriched fraction was aspirated, diluted with eight volumes of HBSS and centrifuged at $250 \times g$ for 10 minutes at 4°C. The cell pellet was washed twice in HBSS by resuspension and centrifugation. A final cell suspension, adjusted to contain 2×10^6 viable lymphocytes per millilitre, was prepared in modified McCoy's growth medium supplemented with 10% heat-inactivated fetal bovine serum. Viable cells were identified by trypan blue dye exclusion and counted.

Mouse blood in 1- to 1.5-ml portions was collected in heparinized tubes by cardiac puncture from groups of 10 to 15 Toximul-treated mice and from age-matched controls. The lymphocyte-rich cell fraction was treated by the Lymphoprep gradient centrifugation method recently described by Crispens[15] for isolating lymphocytes from small volumes of mouse blood. The lymphocytes were washed twice with HBSS and resuspended in McCoy growth medium; the suspension was adjusted to contain 2×10^6 cells per millilitre, as were the suspensions of human lymphocytes.

Interferon production

The stock mouse interferon used in interferon-treatment experiments was produced in monolayer cultures of mouse L-929 cells that were grown in 2.5-l cylindrical roller bottles following induction with the inactivated Newcastle disease virus in multiplicities of infection (MOI) equal to 10.0 and 1.0 plaque-forming units (PFU) per cell.

Assays for the production of interferon by human and mouse lymphocytes were performed in 95-well, flat-bottom microtest plates (Costar, Cambridge, Massachusetts). Each well received 0.1 ml of a cell suspension containing 2×10^5 mouse lymphocytes and 0.1 ml of either McCoy growth medium only (control) or McCoy growth medium containing Newcastle disease virus. Each induced variable was tested six times. The cultures were incubated for 20 hours at 37°C in a humidified incubator containing 4% carbon dioxide. The medium was then collected and acidified to a pH of 2 with normal hydrochloric acid for 96 hours at 4°C. Following neutralization with normal sodium hydroxide the medium was titrated for interferon activity. The method of inducing interferon in human lymphocytes was described previously.[16]

Interferon assay

Assays of human and mouse interferon were carried out in BSC-1 and L-929 monolayer cultures by the plaque reduction technique, with 30 to 50 PFU of vesicular stomatitis virus used as the challenge virus. They were performed in 24-well plates (Costar) and the

results were recorded as 50% plaque reduction doses (PRD_{50})[12] (1 National Institutes of Health unit of interferon = 1.1 to 1.2 Dalhousie PRD_{50} in these assays).

Experiments and results

Evaluation of the effect of emulsifier treatment on serum interferon levels

Two groups of 4-day-old SWR mice were given intraperitoneal injections of 0.5 ml of phosphate-buffered saline containing 0.5 LD_{50} of Toximul MP8 (85 mg/kg). We used intraperitoneal injections rather than our previously reported practice[7] of exposing experimental mice by painting emulsifier on their shaved abdomens because experiments had shown that this change in the method of administering the emulsifier did not affect the virus-enhancement properties of this chemical[10] and did add considerably to the precision with which the effect could be demonstrated. Two other groups of the same number of mice were injected with phosphate-buffered saline, pH 7.2, in the same volume as the emulsifier solution to serve as controls.

After 1, 3, 5, 7 and 10 days the mice in one exposed and one control group were induced to synthesize interferon by the intraperitoneal injection of 100 μg of poly I:C (a polymer of inosine:cytosine). After 5 hours they were killed, their blood was pooled and clotted, and the serum was separated and collected following centrifugation at 4°C. (In previous experiments 5 hours had proved to be the optimum time for determining the amount of interferon induced by poly I:C.)

After the same intervals the mice in the other exposed and the other control group were induced to synthesize interferon by the administration of the inactivated Newcastle disease virus in an amount equivalent to 10^5 PFU. After 16 hours these mice were killed, their blood was pooled and clotted, and the serum was collected by centrifugation.

Interferon assays were performed on the serum from these pools.[11] The results, given in Table I, show that the inhibitory effects of the emulsifier on the production of interferon lasted for at least 10 days after exposure to poly I:C and for 5 days after exposure to inactivated Newcastle disease virus.

Table I—Serum interferon levels in SWR mice exposed to Toximul MP8 and in control mice following interferon induction with poly I:C (two experiments) or inactivated Newcastle disease virus

Time of induction after exposure (d)	Level*; induction agent					
	Poly I:C					
	Exposed mice		Control mice		Newcastle disease virus	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exposed mice	Control mice
1	270	115	810	417	78	128
3	90	140	810	1202	35	210
5	78	ND	417	ND	17	115
7	65	39	510	428	124	96
10	410	857	1150	1445	ND	ND

*Each determination represents the level of interferon, in 50% plaque reduction doses (PRD_{50}), in pooled serum obtained from a group of 5 to 10 mice. ND = not determined.

Evaluation of interferon production by mouse lymphocytes from emulsifier-treated and untreated mice

To evaluate the ability of Toximul MP8 to compromise the induction of interferon by mouse lymphocytes, we divided 7-day-old SWR mice into two groups. The mice in one group were injected intraperitoneally with 0.5 LD_{50} of Toximul MP8 in 0.1 ml of phosphate-buffered saline. After 1 and 3 days 10 to 15 mice from each group were killed, and the lymphocytes prepared from their blood were induced to produce interferon.

The results presented in Table II show a substantial and then a biologically significant reduction in the capacity of lymphocytes from emulsifier-exposed mice to produce interferon, as compared with that of lymphocytes from control mice, when measured 1 and 3 days after treatment.

Evaluation of interferon protection in emulsifier-exposed mice

Three groups of 4-day-old SWR mice were given intraperitoneal injections of 0.5 LD_{50} of Toximul MP8 in 0.1 ml of phosphate-buffered saline. Three control groups of mice of the same age were injected with the diluent only. After 8 days (a period within which maximum inhibition of the interferon response is expected) each mouse in one of the Toximul-treated groups and two of the control groups was injected intraperitoneally with 0.1 ml of phosphate-buffered saline containing 200 PRD_{50} of mouse interferon. Each mouse in the other three groups (two exposed and one control) was given 0.1 ml of saline only. Two hours later four of these six groups — one group that had received two injections of saline, one group that had received saline and then interferon, one group that had received Toximul and then saline, and one group that had received Toximul and then interferon — were each divided into three subgroups. Each subgroup was injected intraperitoneally with encephalomyocarditis virus in a dilution of $\log_{10}$ −7.5, −8.0 or −8.5; these dilutions bracketed the 0.5 LD_{50} for this virus. The other two groups, which had received Toximul or interferon only, now received 0.1 ml of saline.

The survival data are given in Table III. Statistical analysis of the data shows that not only did the emulsifier enhance the virulence of the encephalomyocarditis virus but also it significantly reduced the protective effect of interferon injected 2 hours before the virus (Table IV).

Table II—Interferon production by lymphocytes from SWR mice exposed to Toximul MP8 and from control mice following induction with inactivated Newcastle disease virus

Time of induction after exposure (d)	Interferon level (PRD_{50}); MOI* of virus			
	10.0		1.0	
	Exposed mice	Control mice	Exposed mice	Control mice
1	437	562	166	213
3	18	166	10	138

*Multiplicity of infection.

Evaluation of interferon production by lymphocytes from patients with Reye's syndrome

To determine if lymphocytes from patients in the acute stage of Reye's syndrome were compromised in their ability to synthesize interferon, a protocol similar to that used for the mice was adopted. It differed only in that MOIs of 5.0, 1.0 and 0.5 of Newcastle disease virus were used for interferon induction. In all patients the diagnosis of Reye's syndrome was confirmed in that they met the accepted clinical and biochemical criteria established for this condition.[3,5] In addition, they were considered to have had a related influenza B virus infection because of a significantly high titre (1:128 to 1:512) of complement-fixing antibodies to that virus at the time of study. In five patients blood specimens for lymphocyte studies were obtained during the acute phase of their disease. In three of these patients a blood specimen was also obtained during their convalescence. We also obtained blood from a patient (no. 6) who had not contracted Reye's syndrome but had suffered the same influenza B virus infection as a sibling (patient 2) who had contracted Reye's syndrome. Control lymphocyte cultures were prepared from the blood of this sibling and five healthy individuals. Data describing the ability of the lymphocytes cultured from the blood of the Reye's syndrome patients and the controls to produce interferon following induction by Newcastle disease virus are given in Table V.

Discussion

It is clear that mice exposed to sublethal doses of polyoxyethylene ether-based emulsifiers have an enhanced susceptibility to some virus infections[6,7] and an impaired capacity to benefit from the protective action of interferon.[11] In addition, such exposure resulted in a reduced capacity of these animals to respond to an induction dose of two widely used inducers of serum interferon as compared with that of healthy control mice. The lymphocytes, usually considered to be a major source of endogenous interferon,[19] were also unable to produce interferon to the same degree in the exposed animals as in the healthy control mice when suitably induced. A logical surmise is that these events are linked and that a compromised interferon response may, in part, cause the enhancement of virus lethality seen in mice following sublethal exposure to these emulsifiers. Other investigators have reported that the viruses causing type 3 mouse hepatitis,[20] influenza A,[21] encephalomyocarditis,[22] herpes simplex type 1, Moloney sarcoma, vesicular stomatitis and Newcastle disease[23] are all more lethal to mice that have had their interferon response inhibited by potent anti-interferon antiserum. It is quite conceivable that mice with an interferon response compromised by other methods, such as exposure to polyoxyethylene ether emulsifiers, also are more susceptible to virus infections, as our study showed for encephalomyocarditis.

The lack of protection afforded to emulsifier-exposed mice by exogenous interferon was not entirely unexpected. Earlier we had shown not only that the production of interferon by cultured mouse cells was inhibited by this emulsifier, but also that the cells' ability to respond to exogenous interferon was reduced.[10,11] From the data from this study we conclude that these emulsifiers affect the interferon response doubly: they

Table IV—Comparison* of survival data for groups of SWR mice injected with combinations of phosphate-buffered saline (PBS), Toximul MP8 (TOX) and interferon (IF), and then with encephalomyocarditis virus (EMC)

	Substances injected					
	PBS + PBS + EMC v. TOX + PBS + EMC		PBS + PBS + EMC v. PBS + IF + EMC		PBS + IF + EMC v. TOX + IF + EMC	
EMC $\log_{10}$ dilution	χ^2	P†	χ^2	P‡	χ^2	P§
–7.5	63.28	0.0001	14.82	0.0005	10.12	0.005
–8.0	46.76	0.0001	9.62	0.005	24.41	0.0001
–8.5	11.96	0.001	4.76	0.05	8.04	0.005

*Analysis by Kaplan–Mayer product method and Mantel–Haenszel chi-square summary procedure.[17,18]
†Virus enhancement demonstrated.
‡Interferon protection demonstrated.
§Interferon action significantly compromised.

Table III—The effect of Toximul MP8 (TOX) or phosphate-buffered saline (PBS) on 4-day-old SWR mice and the protective effect of interferon (IF) or PBS given 8 days later (to 12-day-old mice) against encephalomyocarditis virus (EMC) injected a further 2 hours later

Age of mice at time of injection	Substances injected and $\log_{10}$ dilution of EMC (and no. of mice)														
	Control groups					Exposed groups									
4 d 12 d 12 d + 2 h	TOX PBS PBS	PBS IF PBS	PBS PBS EMC			TOX PBS EMC			PBS IF EMC			TOX IF EMC		
			–7.5	–8.0	–8.5	–7.5	–8.0	–8.5	–7.5	–8.0	–8.5	–7.5	–8.0	–8.5
	(29)	(59)	(15)	(16)	(16)	(23)	(20)	(16)	(13)	(20)	(20)	(17)	(20)	(23)
Days after injection of EMC virus	% of mice surviving														
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	93.7	100	100	100	100	100	100	100	100	100	95.6
2	96.5	100	93.3	81.2	93.7	78.3	65.0	87.5	100	95.0	100	100	100	96.6
3	96.5	100	53.3	37.5	87.5	8.7	40.0	68.7	76.9	85.0	100	94.1	70.0	86.9
4	96.5	100	40.0	37.5	75.0	0	0	62.5	69.2	75.0	100	52.9	55.0	92.6
5	96.5	100	40.0	31.2	68.7	0	0	62.5	69.2	65.0	80.0	41.2	40.0	82.6
6	96.5	100	40.0	31.2	68.7	0	0	56.3	69.2	65.0	80.0	35.3	35.0	73.9

reduce interferon synthesis following infection and they inhibit the ability of animals and cells to respond to interferon, either endogenous or exogenous.

The demonstration that peripheral lymphocytes obtained from patients with acute Reye's syndrome are unable to respond to interferon induction as actively as lymphocytes from the same patients in the convalescent phase of their illness or lymphocytes from healthy individuals is very suggestive. In the one appropriate (matched) virus-infected control subject that we have been able to examine, a sibling of a Reye's syndrome patient, influenza B was not followed by the syndrome and no such inhibition was seen, suggesting that in these children infection with this virus alone is not sufficient to impair the lymphocytes' interferon response. Some additional factor appears to be required. This factor could, of course, be unrelated to the environment and be due to treatment or debilitation associated with Reye's syndrome. However, the known association of an impoverished interferon response with enhanced virulence of some other viral infections must be considered.

We view Reye's syndrome as a failure in the normal recovery process that usually follows an influenza B virus infection. One important factor in recovery from virus diseases is the ability to synthesize and utilize interferon. A failure in this ability, or even its substantial compromise by some external factor, such as an emulsifier, may be a significant feature in the genesis of Reye's syndrome.

We thank L. Johnson and C.L.Y. Lee for their expert assistance in these investigations.

Table V—Interferon production by human lymphocytes from patients with acute and convalescent Reye's syndrome, a sibling of one of those patients and healthy donors following 20 hours' induction with Newcastle disease virus

		Interferon level (PRD_{50});* MOI of virus		
Subject	Diagnosis	5.0	1.0	0.5
Patient				
1	Reye's syndrome			
	Acute	4	ND	4
	Convalescent	151	155	197
2	Reye's syndrome			
	Acute	26	38	14
	Convalescent	91	93	112
	Convalescent	79	53	58
3	Reye's syndrome			
	Acute	91	85	151
	Convalescent	158	251	575
4	Reye's syndrome			
	Acute	55	34	17
5	Reye's syndrome			
	Acute	86	61	29
6	Influenza B	269	437	501
Control				
1	Healthy	162	174	182
2	Healthy	94	108	200
3†	Healthy	288	243	346
	Healthy	209	229	437
4	Healthy	155	240	589
5	Healthy	282	ND	380

*Not determined.
†Two blood specimens, obtained 8 days apart, were studied.

This research was supported in part by grants from the Medical Research Council of Canada (MA-1615) and the National Science and Engineering Research Council of Canada.

References

1. REYE RDK, MORGAN G, BARAL J: Encephalopathy and fatty degeneration of the viscera. A disease entity in childhood. *Lancet* 1963; 2: 749–752
2. COREY L, RUBIN RJ, THOMPSON TR, NOBLE GR, CASSIDY E, HATTWICK MAW, GREGG MB, EDDINS D: Influenza B-associated Reye's syndrome: incidence in Michigan and potential for prevention. *J Infect Dis* 1977; 135: 398–407
3. CROCKER JFS, BAGNELL PC: Reye's syndrome: a clinical review. *Can Med Assoc J* 1981; 124: 375–382
4. LINNEMANN CC JR, SHEA L, KAUFFMAN CA, SCHIFF GM, PARTIN JC, SCHUBERT WK: Association of Reye's syndrome with viral infection. *Lancet* 1974; 2: 179–182
5. NELSON DB, HURWITZ ES, SULLIVAN-BOLYAI JZ, MORENS DM, SCHONBERGER LB: Reye's syndrome in the United States in 1977–78, a non-influenza B virus year. *J Infect Dis* 1979; 140: 436–439
6. CROCKER JFS, OZERE RL, SAFE SH, DIGOUT SC, ROZEE KR, HUTZINGER O: Lethal interaction of ubiquitous insecticide carriers with virus. *Science* 1976; 192: 1351–1353
7. CROCKER JFS, ROZEE KR, OZERE RL, DIGOUT SC, HUTZINGER O: Insecticide and viral interaction as a cause of fatty visceral changes and encephalopathy in the mouse. *Lancet* 1974; 2: 22–24
8. CROCKER JFS, OZERE RL: The incidence and etiology of Reye's syndrome in eastern Canada. In CROCKER JFS (ed): *Reye's Syndrome II*, Grune, New York, 1979: 3–12
9. ROZEE KR, LEE SHS, CROCKER JFS, SAFE SH: Enhanced virus replication in mammalian cells exposed to commercial emulsifiers. *Appl Environ Microbiol* 1978; 35: 297–300
10. LEE SHS, LALTOO M, CROCKER JFS, ROZEE KR: Emulsifiers that enhance susceptibility to virus infection: increased virus penetration and reduced interferon response. *Appl Environ Microbiol* 1980; 40: 787–793
11. ROZEE KR, LEE SHS, LALTOO M, CROCKER JFS: A mouse mode of Reye's syndrome: the effect of emulsifiers (polyoxyethylene ether polymers) on the interferon response. In KHAN A, HILL NO, DORN GL (eds): *Interferon: Properties and Clinical Uses*, Wadley Inst Molecular Med, Dallas, Texas, 1980: 279–289
12. O'SHAUGHNESSY MV, LEE SHS, ROZEE KR: Interferon inhibition of DNA synthesis and cell division. *Can J Microbiol* 1972; 18: 145–151
13. REED LJ, MUENCH H: Simple method of estimating 50 per cent endpoints. *Am J Hyg* 1938; 27: 493–497
14. LEE SHS, EPSTEIN LB: Reversible inhibition by interferon of the maturation of human peripheral blood monocytes to macrophages. *Cell Immunol* 1980; 50: 177–190
15. CRISPENS CG JR: Preparation of lymphocytes from small volumes of peripheral mouse blood. *Experientia* 1979; 35: 833–834
16. LEE SHS, ROZEE KR: Biological characterization of tuberculin-induced human lymphocyte interferon-like substance (PPD-IF). *Ann NY Acad Sci* 1980; 350: 584–595
17. KAPLAN EL, MAYER P: Non-parametric estimations from incomplete observations. *J Am Stat Assoc* 1958; 53: 457–481
18. MANTEL N: Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966; 50: 163–170
19. DE MAEYER-GUIGNARD J: Mouse leukemia: depression of serum interferon production. *Science* 1972; 177: 797–799
20. VIRELIZIER JL, GRESSER I: Role of interferon in the pathogenesis of viral diseases of mice as demonstrated by the use of anti-interferon serum. V. Protective role in mouse hepatitis virus type 3 infection of susceptible and resistant strains of mice. *J Immunol* 1978; 120: 1616–1619
21. HALLER O, ARNHEITER H, LINDENMANN J: Genetically determined, interferon-dependent resistance to influenza virus in mice. *J Exp Med* 1979; 149: 601–612
22. GRESSER I, TOVEY MG, BANDU MT, MAURY C, BROUTY-BOYE D: Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. I. Rapid evolution of encephalomyocarditis virus infection. *J Exp Med* 1976; 144: 1305–1315
23. GRESSER I, TOVEY MG, MAURY C, BANDU MT: Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza viruses. Ibid: 1316–1323