IMMUNE MODULATING EFFECTS OF POLY ICLC

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This presentation will compare immune modulation brought about by interferon with those brought about by interferon inducers, particularly double-stranded RNA. If the only effect of the interferon inducer was the induction of interferon, then we would expect that interferon and its inducers would have the same effect in any given immune phenomenon, be it no effect, enhancement or inhibition. The fact that inducers can elicit a higher level of circulating interferon *in vivo* than is readily obtainable by administering exogenous interferon might modify this expectation in that large amounts of interferon might act differently from small amounts of interferon. However, within this limitation, interferon inducers might be expected to act like interferon. Indeed, there are many instances where this is the case. You will hear of them during this session. One such is the enhancement of natural killer cell activity by interferon and by poly I·poly C.[1]

There are some rather clear-cut differences also between the effects of interferon and poly I·poly C. Drs. McNeil and Fleming have reported that interferon blocks the action of a glycoprotein-stimulating factor that stimulates the production in soft agar of granulocyte-macrophage colonies, whereas poly I·poly C, both *in vivo* and *in vitro*, stimulate such activity.[2] That is, even though poly I·poly C leads to the production of interferon, which is a colony-inhibiting substance, the stimulating activity of the double-stranded RNA itself overcomes such inhibition.

Two groups, at least, have found differences between poly I·poly C and interferon on the graft-versus-host (GvH) reaction, with poly I · poly C enhancing GvH reaction and interferon either inhibiting or having no effect.[3,4] Unfortunately in most other areas of immune regulation, direct comparison is not readily made. While both poly I · poly C and the stabilized derivative of poly I · poly C with polylysine and carboxymethylcellulose (poly ICLC) have been tested for their effect on immunity and antibody formation, as has interferon, the several projects have been done by different people in different laboratories using different antigens and different techniques. Mostly, interferon, both the virus-induced type and immune interferon, has been inhibitory to antibody production, while poly I · poly C and its derivatives have been stimulatory.

Rather than dwell further on these comparisons, I would like to present data that show that the primate-effective interferon inducer, poly I · poly C, complexed with polylysine and carboxymethylcellulose is an effective enhancer of immune reactivity when given with certain weakly effective vaccines.

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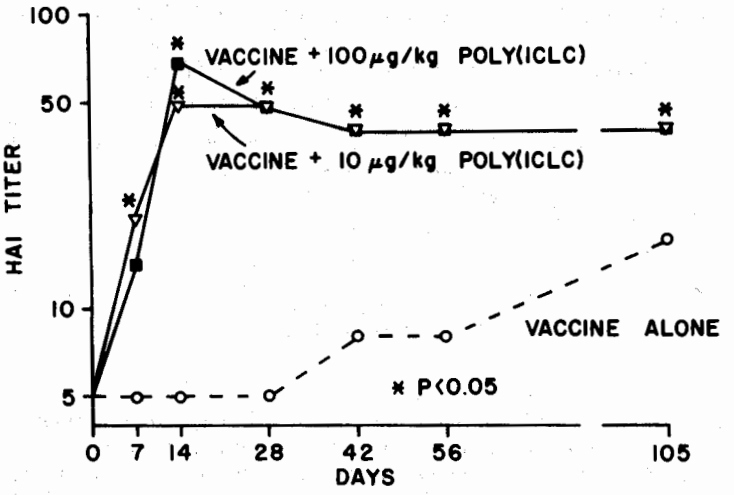

FIGURE 1. Effect of one injection of poly ICLC on HAI antibody production by young Rhesus monkeys in response to a subunit vaccine to swine flu (200 CCA units). (Four monkeys per group)

Monovalent influenza virus subunit vaccine designated A/swine X-53, prepared from A/NJ/76 (New Jersey, Swine) is only moderate to weakly effective when given as a single dose to young people. When the vaccine was given to monkeys simultaneously with one dose of poly ICLC, HAI antibody titers in the serum were detectable earlier and rose to higher levels than in monkeys receiving vaccine alone. Four monkeys were used per group, each receiving 200 CCA units.

The adjuvant activity of poly ICLC was particularly pronounced in young monkeys, where as little as 10 μg of drug per kg of body weight was effective, as seen in FIGURE 1. This level of poly ICLC does not induce detectable levels of serum interferon, and no fever was produced.[5]

Analogous results were obtained in monkeys using inactivated Venezuelan Equine Encephalomyelitis virus vaccine.[6] FIGURE 2 shows some of the data. It can be seen that antibody levels in serum were boosted about 40-fold after primary immunization when one compares levels attained after administration of vaccine along with poly ICLC with that attained with vaccine alone, and perhaps 200-fold after a secondary immunization. There was no alteration in the progression of IgM and IgG development. At the peak of antibody levels, most of the antibody was IgG. Polylysine complexed to carboxymethylcellulose without poly I · poly C had no adjuvant action.

A polysaccharide vaccine made from *Hemophilus influenzae* is a poor vaccine in very young children, where the disease threat is maximum. The vaccine is also poor in young monkeys. TABLE 1 shows this.[7] The data presented are normalized values, obtained by radioimmune assays done by Dr. Porter Anderson. The value of 100 was assigned in each case to the amount of radioactivity found prior to immunization. The vaccine alone caused a minimum boost, but when given with poly ICLC there was a more pronounced boost.

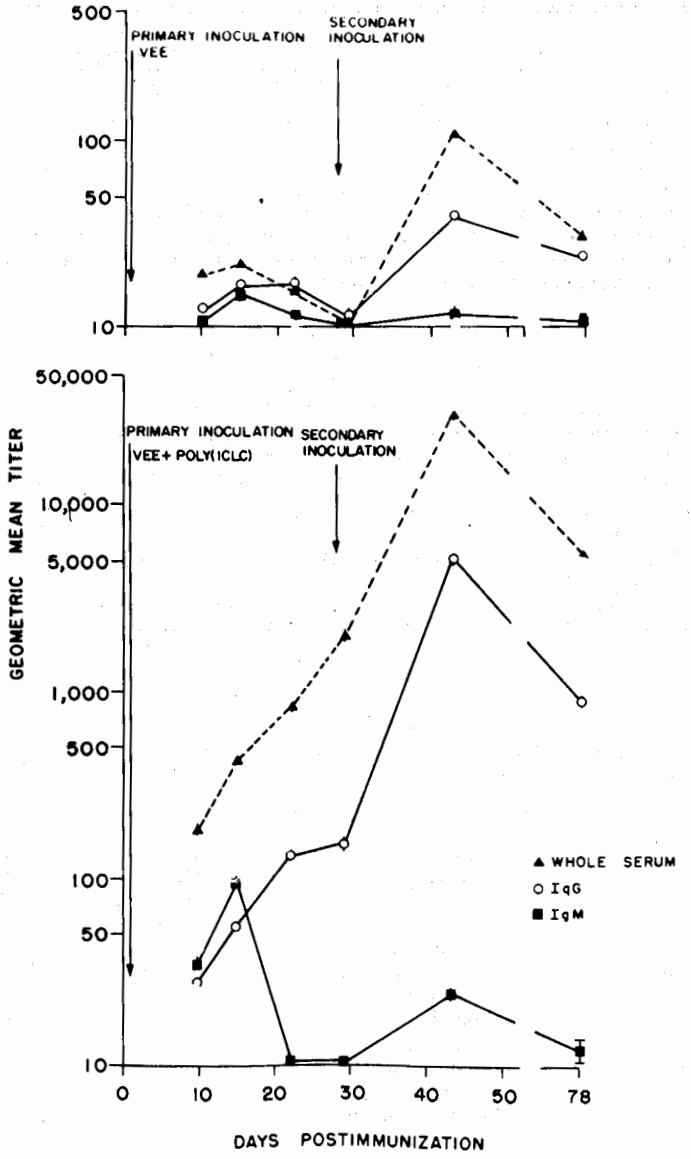

FIGURE 2. Effect of poly ICLC (200 μg/kg body weight) on antibody production by Rhesus monkeys in response to Venezuelan equine encephalomyelitis (VEE) virus vaccine (strain tc-83). (Four monkeys per group)

TABLE 1

EFFECT OF ADJUVANTS ON RESPONSE TO *HEMOPHILUS INFLUENZAE* VACCINE*

Treatment	Pre	Antibody Levels, (normalized ng/ml) Day 18	Day 25	Day 32
Vaccine dose	100	114	124	143
PICLC + (1 mg/kg) Vaccine	100	564	435	300
PAULC	100	191	166	166

*Comparison of effects of poly ICLC and polyadenylic-polyuridylic acid-polylysine carboxymethylcellulose on antibody production by Rhesus monkeys in response to a polysaccharide vaccine for *Hemophilus influenzae*.

TABLE 2

EFFECT OF LOW DOSES OF POLY ICLC ON ANTIBODY PRODUCTION BY RHESUS MONKEYS IN RESPONSE TO A POLYSACCHARIDE VACCINE FOR *HEMOPHILUS INFLUENZAE*

Treatment	Pre	Day 7	Day 14	Day 20	Day 28	Day 34	Day 42
Vaccine	100	590	348	286	225	187	113
Vaccine + poly ICLC, 0.3 mg/kg	100	5643	5040	3340	2063	2162	780
Vaccine + poly ICLC, 0.03 mg/kg	100	6589	3904	1884	1132	839	721

TABLE 3

EFFECTS OF ADJUVANTS ON SURVIVAL OF VACCINATED MICE*

Treatment				% Survivors Day 35
Vaccine		Adjuvant	Dose/kg	(N = 16)
Vaccine	+	Poly (ICLC)	20 μg	50
			100 μg	50
			200 μg	13
Saline	+	Poly (ICLC)	200 μg	6
Vaccine	+	Freund's complete adjuvant		6
Saline	+	Freund's complete adjuvant		6
Vaccine	+	Freund's incomplete adjuvant		6
Saline	+	Freund's incomplete adjuvant		0
Vaccine controls				19
Saline controls				0

*Effects of one dose of poly ICLC, Freund's complete adjuvant, or Freund's incomplete adjuvant plus vaccine vs. Rift Valley Fever (Zagazig) virus on survival of mice challenged on day 14 postimmunization with 750 PFU virulent virus.

Polyadenylic · polyuridylic acid complexed to CMC and polylysine was not so effective as poly ICLC.

TABLE 2 show another experiment of the same type where the dose of poly ICLC was reduced.[7] There was no statistical difference between the two doses. In three experiments there was no effect of poly ICLC on platelets, total and differential leucocytes, packed cell volume, erythrocyte sedimentation rate, serum urea nitrogen, total and direct bilirubin, glutamicoxalacetic transaminase, latic dehydrogenase, alkaline phosphatase, and creatinine.

In an experiment using a different type of endpoint, it was shown that poly ICLC, given together with a vaccine versus Rift Valley fever (RVF) virus, increased the survival of mice subsequently challenged with live RVF virus.[8] TABLE 3 shows the increased survival rates of mice given poly ICLC and vaccine, as compared with those found in mice immunized with vaccine alone.

The last of these adjuvant effects that I will mention deals with observations made with Joachim Hilfenhaus, using an envelope antigen made from herpes virus.[9] In these experiments, both antibody levels and survival after subsequent

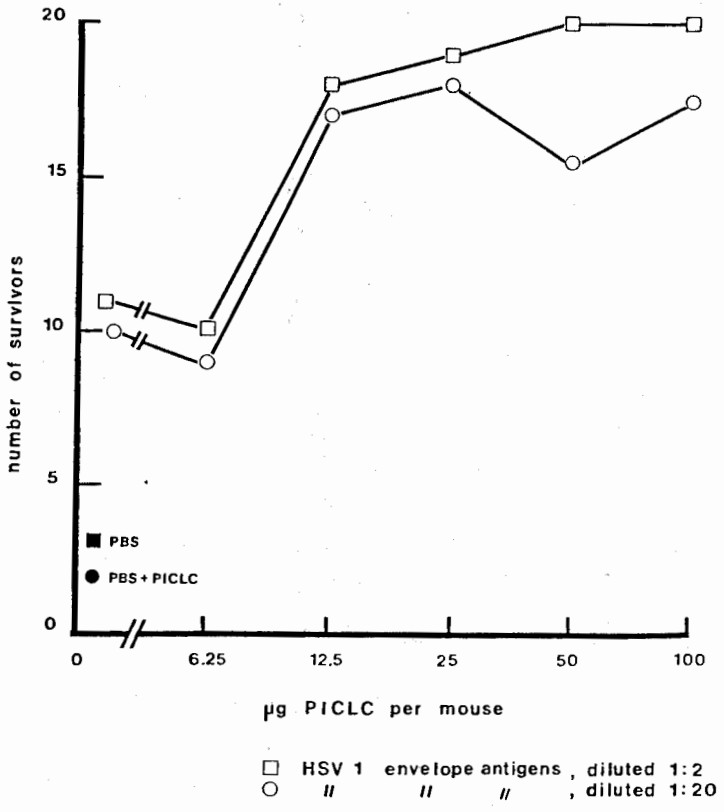

FIGURE 3. Effect of poly ICLC given with envelope antigen from herpes virus on survival in mice against subsequent challenge with live virus.

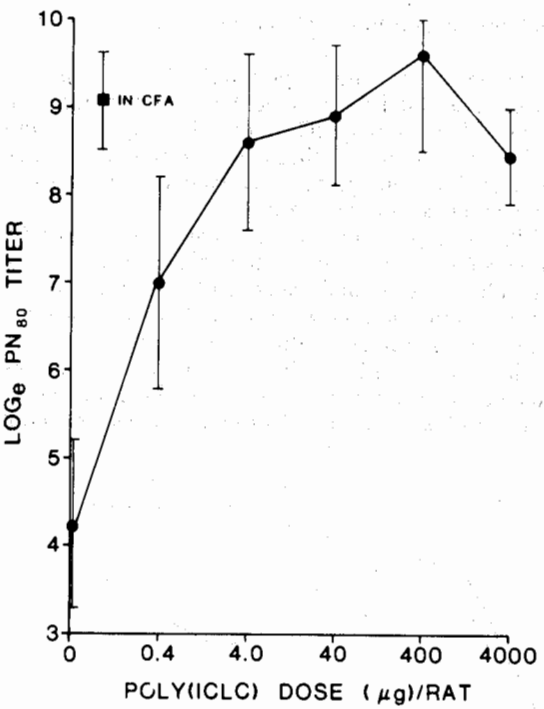

FIGURE 4. Effect of poly ICLC on antibody production by rats in respose to Venezuelan equine encephalomyelitis virus vaccine.

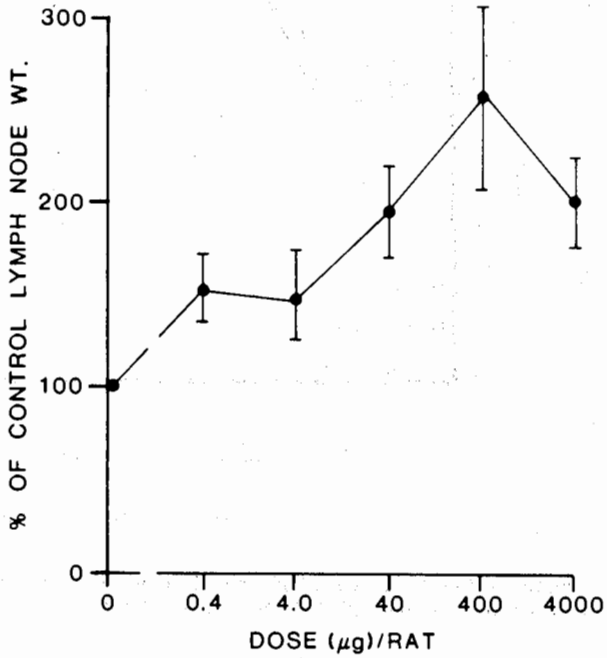

FIGURE 5. Effect of dose of poly ICLC given along with VEE virus vaccine on weight of lymph nodes draining the site of injection.

challenge with whole virus were measured. Both these aspects of immunity were enhanced when the envelope antigen was administered with poly ICLC. FIGURE 3 summarizes some of the data.

Dr. Arthur Anderson has done some experiments in rats that shed some light on physiological changes associated with administration of antigens plus poly ICLC.[10]

FIGURE 4 shows that antibody response by rats to Venezuelan equine encephalomyletis vaccine is augmented by poly ICLC as it is in monkeys.

FIGURE 5 relates that the weight of the nodes draining the area where the poly ICLC was injected increases as the dose of drug increases. With 40 μg/rat there is an increase of 100 percent.

FIGURE 6 shows that the number of small lymphocytes in the high venules of

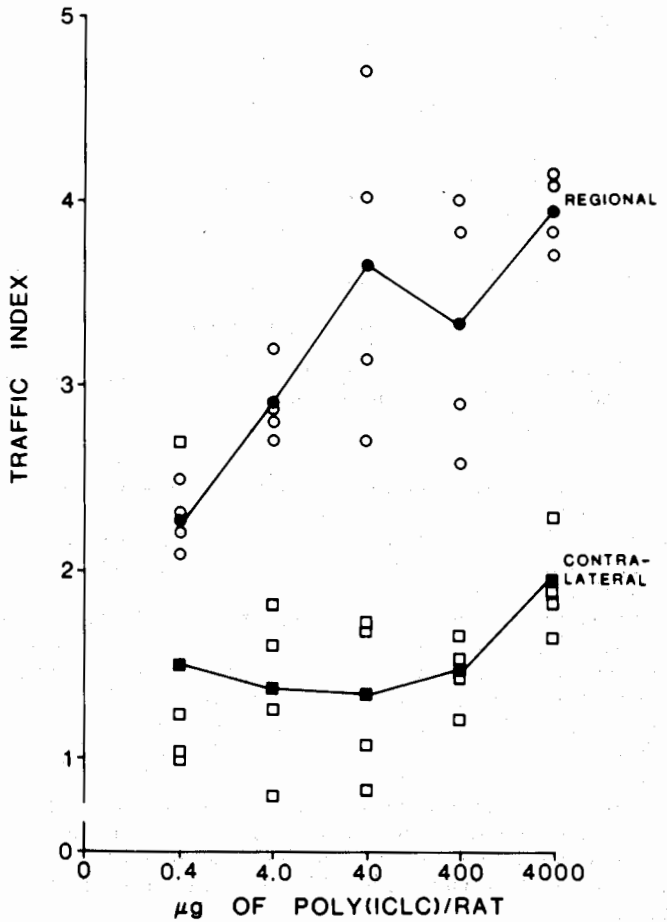

FIGURE 6. The lymphocyte migration index in the high venules of the paracortex of the nodes mentioned in FIGURE 5.

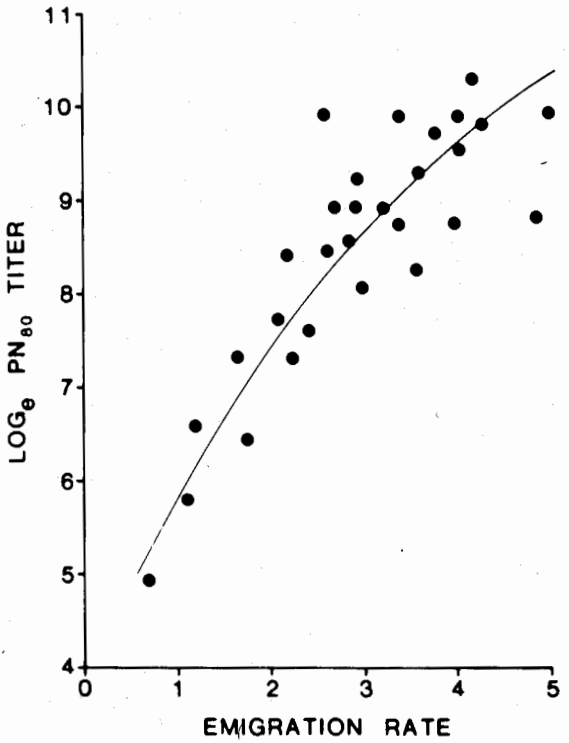

FIGURE 7. Correlation of the number of lymphocytes in the paracortex of nodes 2 days after injection, with antibody levels 28 days after infection.

the paracortex of these nodes increases with the dose of poly ICLC within 24–48 hours after injection.

Finally, to complete the picture, FIGURE 7 shows that in individual rats the number of small lymphocytes in the paracortex at 2 days relates to the antibody level in that animal at 28 days.

It is therefore apparent that, somehow, injection of poly ICLC increases the size of the node, this increase in size is associated with an increase in small lymphocytes, which are cells capable of producing antibody, and the increase in number of these lymphocytes is related to the increase in antibody production.

One other thing should be mentioned, because it ties in in ways that seem related. Poly ICLC is a good radioprotective agent.[11] Mice given poly ICLC can tolerate and recover from a significantly higher level of x-irradiation than mice not given the inducer. This protection and recovery is associated with an increase in the number of growing stem-cell colonies in spleen and marrow. Which brings us back to where we started, namely, that double-stranded RNA does seem to enhance growth of certain white cells, which is a different effect from that seen with interferon.

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