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Polyinosinic Acid-Polycytidylic Acid and Its Mismatched Analogues: Differential Effects on Human Cell Function

JUDITH A. O'MALLEY, SUSAN S. LEONG, JULIUS S. HOROSZEWICZ, AND WILLIAM A. CARTER

Department of Medical Viral Oncology, Roswell Park Memorial Institute, Buffalo, New York 14263

JAMES L. ALDERFER[1] AND PAUL O. P. TS'O

Division of Biophysics, Department of Biochemical and Biophysical Studies, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

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SUMMARY

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This study extends, into human systems, earlier work with mismatched analogues of polyinosinic acid-polycytidylic acid, $rI_n \cdot rC_n$, which were consistently shown to be less toxic than $rI_n \cdot rC_n$ in various lower animal systems. We studied the mismatched analogues in a variety of human cells, hoping thereby to obtain an index of possible therapeutic efficacy and toxicity which might be observed in intact man. At doses which induce antiviral activity, the mismatched analogues of $rI_n \cdot rC_n$ do not detrimentally affect various cell systems such as the cloning of human myeloid colony forming cells. With suspensions of human splenocytes, mismatched inducers are much less mitogenic than $rI_n \cdot rC_n$, and the mismatched inducers show less effect on retarding the growth of human fibroblastic cells.

INTRODUCTION

The induction of interferon biosynthesis by synthetic and naturally-occurring double-stranded (ds)[2] polyribonucleotides has been well documented (1-3). Polyinosinic·polycytidylic acid, $rI_n \cdot rC_n$, being readily available and one of the most active, thus has been studied extensively. In addi-

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[1] Current address: Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14263.

[2] The abbreviations used are: dsRNA, double-stranded RNA; $rI_n \cdot rC_n$, polyinosinic acid·polycytidylic acid duplex; $rI_n \cdot r(C_{12},U)_n$, mismatched polynucleotide duplex containing unpaired uracil bases located in the polycytidylic acid strand; $rI_n \cdot r(C_{29},G)_n$, mismatched polynucleotide duplex containing unpaired guanine bases located in the polycytidylic acid strand; VSV, Vesicular stomatitis virus; NDV, Newcastle disease virus; PHA, phytohemagglutinin; CFC, human myeloid colony-forming cells; moi, multiplicity of infection.

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tion to its strong antiviral activities, $rI_n \cdot rC_n$ also displayed a wide range of toxicities; the toxicities vary significantly across different animal species (3). The first detailed pharmacologic effects of $rI_n \cdot rC_n$ in man have only recently been reported (4, 5), and the results emphasize the need for interferon inducers with greater medical efficiency. They suggest the possible value of increased inducer dosage in man, which is at present limited at 6-9 mg/kilo (body weight) for $rI_n \cdot rC_n$, due to its toxicity (4).

Carter *et al.* have previously studied the sensitivity of the induction mechanism to subtle alterations in the $rI_n \cdot rC_n$ molecule (6). When bases which do not form a Watson-Crick base pair with hypoxanthine, such as uracil (U) or guanine (G), were inserted into the $r(C)_n$ strand, the resultant $rI_n \cdot r(C, \text{ plus G or U})_n$ duplex contained mismatched regions which probably have an irregular (perhaps "loopout") structure (Fig. 1). A detailed structure·function study indicated that the antiviral activity of these mismatched inducers in human neonatal fibroblasts was retained, provided that the frequency of U and G substitution did not exceed one residue in 12 or 20, respectively. Since $rI_n \cdot r(C_{13},U)_n$ and $rI_n \cdot r(C_{20},G)_n$ were hydrolyzed enzymatically 5-8 times faster than $rI_n \cdot rC_n$ (6), prolonged maintenance of the intact double helical structure was definitely shown not to be a prerequisite for "triggering" interferon production. Indeed, it was proposed that prolonged maintenance of an intact double helical structure actually might lead to the other various responses, commonly scored as drug toxicities (2, 6). Subsequent studies with thiolated polyribonucleotides have also supported the notion that inducer resistance to nucleases is certainly not crucial to induction (7).

Recent work in various animal systems has consistently shown the mismatched analogues to be less toxic than $rI_n \cdot rC_n$: particularly prominent have been greatly reduced pyrogenicity in rabbits (≥100-fold), as well as lethality and mitogenicity in the mouse, while antiviral activity remained constant (8). In a parallel study, which also included a naturally-occurring dsRNA, designated BRL 5907, the polynucleotide duplexes were further tested in mice in a repetitive dose study (9). Effects on body weight, cellular components of blood, spleen and thymus, as well as antigenic properties of the inducers were evaluated, all using coded samples to eliminate possible investigator bias. The order of capacity of these dsRNAs to trigger specific secondary or toxic responses was established as: BRL 5907 $\simeq rI_n \cdot rC_n > rI_n \cdot r(C_{29},G)_n \gg rI_n \cdot r(C_{12},U)_n$.

The above findings clearly suggested that therapeutic gains in man might be achieved with mismatched inducers, particularly $rI_n \cdot r(C_{12},U)_n$. Therefore we embarked on a two pronged course: first, to investigate in a variety of human cell systems the activity of mismatched inducer vis-à-vis $rI_n \cdot rC_n$, hoping thereby to obtain some index of drug efficacy as well as possible clues to the pathogenesis of certain drug-induced lesions already seen in man (4); second, to prepare a quantity of $rI_n \cdot r(C_{12},U)_n$ sufficiently large for its clinical evaluation.

We report herein studies with various human cells, including some of highly specialized function, which are apparent targets in man when $rI_n \cdot rC_n$ or human interferon preparations are administered. Our studies suggest that certain lesions reported in man with both inducers and relatively impure interferon preparations may not be due to the interferon molecule.

MATERIALS AND METHODS

1. *Polynucleotides*

rI_n and rC_n were obtained from Miles Laboratories. Preparation and charactertization of the mismatched polymers and $rI_n \cdot rC_n$ have been described in detail previously (6).

2. *Cells*

A. Human peripheral blood leukocytes. Buffy coats from plasmapheresis donors were mixed with 10 units/ml of preservative-free heparin and 10% v/v plasma gel (R. Bellon Laboratories, Neuilly, France). Leukocyte-rich plasma was obtained after the erythrocytes were allowed to settle for 45 min at room temperature. The cells were washed once by centrifugation (200 × *g* for 10 min) and resuspended at a concentration

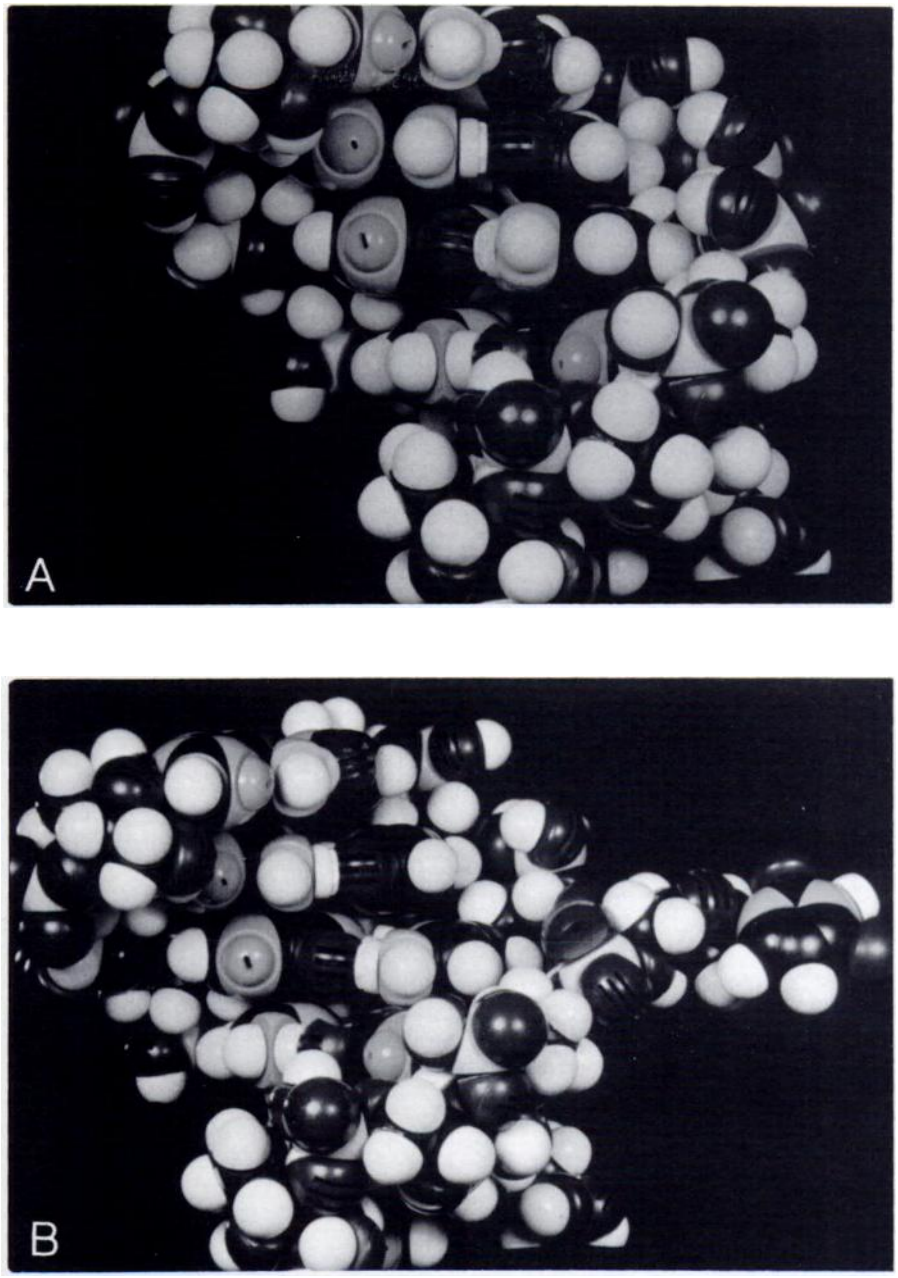

FIG. 1. *Space filling models of typical regions of completely base-paired dsRNA [panel A, $rI_n \cdot rC_n$] and a mismatched dsRNA [panel B, $rI_n \cdot r(C_{12}U)_n$]*

In the mismatched polymer pair, the unpaired base is seen protruding into the solvent, thereby facilitating nucleolytic attack. The exact point of nucleolytic attack has not been identified.

of 8×10^6 cells/ml in medium 1641 enriched with 8% fetal calf serum, 8% human serum, 7% trypticase soy broth and also containing 12 units heparin/ml (12). Aliquots of this suspension (10) were mixed with the various duplex polymers and incubated for 1 hr at 37° in a 5% CO_2 atmosphere (8). The cells were then examined for:

1. Interferon production: Following incubation with the polymers, the cells were

washed, re-suspended in medium 1641 containing 5% fetal calf serum, and incubated for 18 hr at 37° in a 5% CO_2 atmosphere. The cells were centrifuged and the supernatant was then harvested for interferon assay, the procedure for which has been described previously (6, 11). For viral-induced leukocyte interferon, cells were mixed with NDV (moi = 10) for 1 hr at 37° in a 5% CO_2 atmosphere. Cells were then washed, resuspended in 2.5-fold their original volume, and incubated a further 18 hr. Leukocytes were then sedimented and the supernatant harvested; the supernatant was acidified to pH 2 for 72 hr by perchlorate treatment to inactivate residual virus. All interferon levels are expressed as the geometric mean of two or more assays and are stated in Reference Units (Reference Standard 69/19 was used).

2. Myeloid colony formation: These procedures are described in detail elsewhere (12, 13). Briefly, following incubation with polymers, 0.8 ml aliquots of the reaction-mixture were diluted 10-fold with complete growth medium, and plated over "feeder layers." The colonies were counted after 14 days of incubation at 37°. The results are presented as the absolute number of colonies obtained from 4×10^5 leukocytes plated in sextuplicate as well as the percentages of growth in comparison to the controls. Statistical treatment is also described (12).

3. Human mitogen response: Leukocyte suspensions were treated with phytohemagglutinin (PHA, 1 μg/ml, Burroughs-Wellcome Labs) and incubated at 37° for 72 hr. $rI_n \cdot rC_n$ or its mismatched analogues, at various concentrations, were added to the cells and incubated 3 hr. [^{3}H]thymidine (specific activity, 6.7 C/mmole, Schwarz/Mann), was added and the cultures incubated for 16 additional hr, and processed. Data are presented as described elsewhere (8).

B. Human spleen cells. Spleen specimens obtained at surgery were minced, suspended in McCoy's medium at 2×10^6 cells/ml, and incubated with $rI_n \cdot rC_n$ or its mismatched analogues at 37° for 30 min, with and without 16% human serum (8).

Mitogen response: The cultures were then diluted with growth medium and adjusted such that the serum concentration in all suspensions was 10%. The cultures were incubated for 40 hr, pulsed with 1 μC of [^{3}H]thymidine per culture for 6 hr, and processed for scintillation counting as previously described (8).

C. Human foreskin fibroblasts. Preparation of human foreskin fibroblast cultures was carried out as described previously (14). After monolayers of the cells were established (2×10^4 cells/well), the cells were treated for 1 hr with $rI_n \cdot rC_n$ or its mismatched analogue (at concentrations of 10 μM and 100 μM) every 48 hr through day 8. Following each treatment, fresh growth medium was added. In all instances. for growth of these cells, human serum (10%) was substituted for the calf serum routinely used (14); we reasoned that the human serum—being a source of nuclease activity—would mimic some of the dsRNA degradative mechanisms at play, as when $rI_n \cdot rC_n$ is used *in vivo* (4).

RESULTS

Previously, we have shown that $rI_n \cdot rC_n$ and its mismatched analogues induce similar antiviral activity in human fibroblasts (6). We now extend these observations to human leukocytes which are believed to be a major source of interferon *in vivo* (15). As shown in Table 1, a wide range of interferon induction activity (0–190 ref. U/ml) was found when leukocytes derived from several donors were treated. All of the polymers displayed some activity (except in donor 3), although there is a suggestion that $rI_n \cdot r(C_{29},G)_n$ was less active than the others. Apparently the percent lymphocytes in each set of leukocytes does not seem to be a major factor in the amount of interferon produced (10). A more uniform level of response was seen with the viral inducer, NDV. The higher interferon level observed with NDV, rather than synthetic inducers, has been previously observed with human leukocytes *in vitro*. The titers of interferon induced here are several fold lower than those previously with NDV (16) and may reflect the shorter time (1 hr vs. 24 hr) of cell exposure to virus before washing.

We then evaluated certain aspects of cel-

TABLE 1

Interferon induction by $rI_n \cdot rC_n$ and its mismatched analogues in human leukocytes

Details are presented in MATERIALS AND METHODS. Incubation with the buffer alone gave no measurable interferon in any of the six leukocyte donors.

Leukocyte donor	Interferon inducer				% Lymphocytes
	$rI_n \cdot rC_n$ 5×10^{-4} M	$rI_n \cdot r(C_{12},U)_n$ 5×10^{-4} M	$rI_n \cdot r(C_{29},G)_n$ 5×10^{-4} M	NDV moi = 10	
1	18	<5	<5	120	N.D.[a]
2	180	70	190	240	N.D.[a]
3	<5	<5	<5	160	34%
4	44	40	<5	190	44%
5	19	12	<5	105	49%
6	18	30	11	260	50%
7	20	<5	<5	250	61%
8	22	18	8	460	30%

[a] N.D., not done

lular toxicity which, based on earlier clinical studies, might become the limiting factor in the eventual application of mismatched inducers.

Particularly prominent in administration of human leukocyte interferon preparations in man has been a uniform depression in white blood cell count (17), which usually reflects a decrease in precursor cells in the myeloid series. Indeed, direct addition of these interferon preparations to the agar cultures of myeloid precursors results in a very toxic response (18). The problem in man is less when inducers, rather than interferon preparations, are administered (4, 5). We studied the effects of mismatched inducers on human myeloid colony-forming cells (CFC) at concentrations (500 μM and 20 μM) which are effective for induction of antiviral activity, and probably higher than those which can be reached *in vivo*. Human peripheral leukocytes, which normally contain CFC, were first treated with $rI_n \cdot rC_n$ and its mismatched analogues for 1 hr at 37°; the reaction mixtures were then diluted with growth medium and plated over "feeder layers." After 14 days, colony formation was evaluated. The results (Table 2) show that the ability of myeloid precursors to form colonies is not impaired by either $rI_n \cdot rC_n$ or mismatched polymers. Detailed control experiments (data not shown) were also done which indicated that there was no 'entrapment' of the inducer within soft agar. We conclude that, at least *in vitro*, CFC suppression is not seen with

TABLE 2

Effect of $rI_n \cdot rC_n$ and the mismatched analogues on human myeloid colony-forming cells

Details are presented in MATERIALS AND METHODS.

Polynucleotide duplex		Number of colonies		% of growth in controls	
		Mean	±SD	Mean	±SD
$rI_n \cdot rC_n$	500 μM	44.2	6.0	114	13.6
	20 μM	40.7	3.6	105	8.8
$rI_n \cdot r(C_{13},U)_n$	500 μM	40.8	6.4	106	15.7
	20 μM	37.7	5.5	97	14.6
$rI_n \cdot r(C_{29},G)_n$	500 μM	40.0	7.8	103	19.5
	20 μM	42.7	5.9	110	13.8
Controls		38.7	7.3	100	18.8

interferon inducers; this observation is consistent with the suggestion (19) that other mediators, present in crude leukocyte interferon preparations, can also give *in vivo* effects (see DISCUSSION).

We also examined two aspects of an immunologic response which might be modulated by dsRNAs: first, their intrinsic mitogenic potential (Table 3) as measured directly on fresh human splenocytes; and second, their ability to modulate a blastogenic response which has already been initiated by another mitogen (Table 4). The dsRNA effects on various limbs of the immune response are well documented in animal systems (20, 21), in particular, the ability of dsRNA to either enhance or abort a blastogenic response.

Table 3 shows the influence of mismatched base pairs on the mitogenic properties of dsRNA on human splenocytes.

TABLE 3

Effect of $rI_n \cdot rC_n$ and its mismatched analogues on mitogenicity of human spleen cells

Experimental details are presented in MATERIALS AND METHODS.

Polynucleotide duplex	Concentration	With serum		No serum	
		Average CPM	Stimulation index	Average CPM	Stimulation index
$rI_n \cdot rC_n$	330 μM	5,010	1.6	7,461	2.7
	33 μM	3,340	1.0	3,267	1.2
$rI_n \cdot r(C_{12},U)_n$	330 μM	4,100	1.3	3,436	1.2
	33 μM	3,150	1.0	3,395	1.2
$rI_n \cdot r(C_{29},G)_n$	330 μM	3,970	1.2	3,336	1.2
	33 μM	2,840	0.9	2,779	1.0
Buffer A		3,220	1.0	2,806	1.0
PHA		18,600	5.8	16,259	5.8

TABLE 4

Effect of $rI_n \cdot rC_n$ and its mismatched analogues on [^{3}H]-thymidine uptake in PHA-stimulated human leukocytes

Experimental details are presented in MATERIALS AND METHODS.

Polynucleotide duplex	Conc.	CPM incorporated	% of controls
$rI_n \cdot rC_n$	10^{-4} M	10,200	97
	10^{-5} M	9,770	93
	10^{-6} M	11,100	105
	10^{-7} M	9,570	91
$rI_n \cdot r(C_{20},G)_n$	10^{-4} M	11,100	106
	10^{-5} M	11,100	106
	10^{-6} M	10,300	98
	10^{-7} M	9,870	94
$rI_n \cdot r(C_{12},U)_n$	10^{-4} M	11,300	107
	10^{-5} M	11,800	112
	10^{-6} M	11,900	113
	10^{-7} M	10,800	103
IMP & CMP equimolar	10^{-4} M	11,600	110
	10^{-5} M	12,400	117
	10^{-6} M	11,300	107
	10^{-7} M	11,700	111
Cytosine arabinoside	25 μg/ml	680	6.4
Cycloheximide	50 μg/ml	656	6.2
Unstimulated (no PHA)		156	1.5
Control (Buffer + PHA)		10,500	100

Cell suspensions, obtained from a surgical specimen, were first incubated with the compounds in the presence and absence of 16% human serum, cultivated and then pulsed with [^{3}H]-TdR for 6 hr, and relative amounts of incorporation were determined. Buffer A served as a negative control by providing a background value of [^{3}H]thymidine incorporation, and treatment with phytohemagglutinin served as a positive control in demonstrating the viability and responsiveness of these cells in culture (8). $rI_n \cdot rC_n$ (~300 μM) had a stimulation index (last column, Table 3) approximatley one-half that of the potent plant mitogen, PHA; a dose-response effect was suggested with $rI_n \cdot rC_n$, although the overall magnitude of the stimulation index is several-fold less than observed in murine splenocytes (8). Importantly, the two mismatched inducers at identical concentrations demonstrated little or no mitogenic potential. Assuming a linear extrapolation in dosage, these two mismatched inducers may be 10-fold less active than $rI_n \cdot rC_n$ at a high dosage level. Such a differential effect might also be seen in man with circulating RNA duplexes gaining access to splenocytes within the reticuloendothelial network (see DISCUSSION).

Experiments illustrated by Table 4 were designed to determine any modulatory effects of dsRNAs on a PHA directed mitogenic response of human leukocytes. Specifically, following $rI_n \cdot rC_n$ treatment, a transient impairment in lymphocyte response to mitogens has been often noted in man (5), although apparently the impairment in lymphocyte DNA synthesis is not a constant clinical finding. For our *in vitro* analysis with several types of dsRNA, we also included well-established antimetabolites, cytosine arabinoside and cycloheximide as positive controls to demonstrate that the PHA-induced human leukocyte response could definitely be aborted. Table

4 shows that PHA induces a 2 log increase in apparent DNA synthesis which can be blocked about 95% by either cytosine arabinoside or cycloheximide. We also found that equimolar amounts of mononucleotides, IMP and CMP, as might be produced by the expected hydrolysis of the added polynucleotide duplexes, did not influence DNA synthesis (Table 4, 4th experimental set). The three polynucleotide duplexes were then studied over a wide concentration range, 100 μM to .1 μM. Surprisingly, no modulation of [^{3}H]thymidine uptake was seen with any of the duplexes, including $rI_n \cdot rC_n$ at 100 μM. Therefore, we conclude that the transient impairment noted *in vivo* (5) may either be a unique property of certain batches of $rI_n \cdot rC_n$ or that the cells which participate in this impairment *in vivo* are absent in our preparations.

Given the evidence that dsRNAs may have cell-regulatory activity in animals (2), we also investigated possible antiproliferative action which the modified duplexes might have on human fibroblastic cells. In particular, it has been recently noted that highly purified mouse interferon can exert an antiproliferative effect on mouse fibroblasts (22). The experiment (Fig. 2) with human cells was set up in the following manner: different sets of cells were treated with the various inducers every 2 days for 1 hr in serum-free medium. The medium containing the inducer was then decanted, cell sets washed, and fresh medium containing 10% human serum was added. For the first 48 hr after polymer exposure, no effects were seen on cell growth (Fig. 2); thereafter, a progressive fall in cell number was seen at the high dose (100 μM) of $rI_n \cdot rC_n$. Thus, by day 6, control cultures contained twice as many cells as those treated with $rI_n \cdot rC_n$ (100 μM). At a 10-fold lower dose, the $rI_n \cdot rC_n$ effect on cell division was not seen until day 6, suggesting that it may be the "build-up" of residual polymer which ultimately results in a reduced growth rate. In the case of $rI_n \cdot r(C_{13},U)_n$, it apparently requires a longer time to exert a measurable effect on growth of these human fibroblastic cells, as the onset of measurable suppression was delayed until day 6 to 8 (Fig. 2). The difference in response curves for $rI_n \cdot r(C_{13},U)_n$ at 10^{-5} M or 10^{-4} M was not statistically significant. Since we have shown that these cells respond identically, in terms of interferon biosynthesis, to both $rI_n \cdot rC_n$ and its mismatched congeners (6, 8), one can conclude that the differential effects on cell growth may be independent of the interferon molecule *per se*. Namely, the effects reflect additional cell-directed properties of dsRNAs, aside from interferon induction.

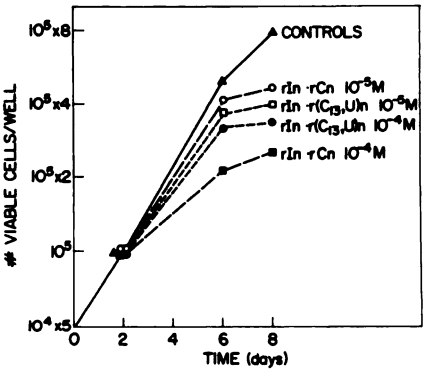

FIG. 2. *Cytotoxicity of $rI_n \cdot rC_n$ and $rI_n \cdot r(C_{13},U)n$ on human foreskin fibroblasts*

Human foreskin fibroblasts were seeded at a density of 2×10^4 cells per well. After 24 hr, the cells were treated with $rI_n \cdot rC_n$ or $rI_n \cdot r(C_{13},U)_n$ at 10^{-4} or 10^{-5} M in serum-free medium for 1 hr. They were then incubated in medium containing 10% human serum. This procedure was repeated every two days. Each point represents an average of two or more wells.

DISCUSSION

The strategy in designing a polynucleotide duplex of greater efficacy rests on the basic premise that either the structural or the temporal requirements of the desirable and the undesirable responses are not identical. This paper represents the fourth report of our long-term program designed to determine if a superior, and clinically useful, interferon inducer can be developed on the basis of emphasizing the probable differences in temporal requirements between the antiviral and other biological responses induced by dsRNAs.

The current text focuses on possible differential effects seen with various cells of exclusively human origin. Effects on cells with highly diverse differentiation potential were explored. Some of our experiments

were focused on specific toxicity of certain cells because of current clinical data with interferon preparations which suggested them to be unusually sensitive targets.

We first found that fresh human leukocyte preparations, when exposed to mismatched inducers, generally do initiate interferon production.

Leukocytes are known to respond less efficiently to synthetic dsRNAs than to viral agents *in vitro* (23), although *in vivo* leukocyte interferon production is seen quickly after induction (15).

We also report here that a completely base-paired inducer, $rI_n \cdot rC_n$, has mitogenic activity with human lymphocyte preparations, thus extending a previous report in murine splenocytes (8). Importantly, the two mismatched inducers had little or no mitogenic activity, even at concentrations which should greatly exceed any of those expected *in vivo*. This reduced arousal of the immune system, as judged by direct specific mitogenic effects, might prove to have important clinical implications. Namely, it is known that $rI_n \cdot rC_n$ can easily activate latent autoimmune disease in the mouse (24), and, if similar immunologic features were also observed with mismatched inducers in animal models, their clinical application would seem to be restricted only to life-threatening disease.

We also examined another limb of the human immune response, namely, the possible ability of synthetic inducers to modulate a blastogenic response once it has been initiated by a plant lectin (PHA). Clinically, there is a suggestion of transient impairment of lymphocyte responsiveness following $rI_n \cdot rC_n$ administration (5), but we were unable to confirm this phenomenon by *in vitro* studies with three different inducers at various concentrations.

A prominent feature of the clinical toxicity with human leukocyte interferon preparations has been depression of white blood cell count (17), which might reflect a decrease in precursor cells of the myeloid series. Thus, we determined directly the effects of mismatched inducers on human myeloid colony-forming cells. In the presence of any of the polynucleotide inducers ($rI_n \cdot rC_n$ or the two mismatched polymer pairs), the proliferation of myeloid clones in soft agar was completely normal. In other experiments[3] we have found that high concentrations of purified fibroblast interferon—over 550 units/ml—are required to suppress myeloid CFC by 50%. Therefore, the effects observed *in vivo* on marrow function may be related to other products in the leukocyte interferon preparations. Both viral (used as inducer) and chicken proteins are known to be present in such preparations (25). Human fibroblast interferon (26) induced by $rI_n \cdot rC_n$ and purified several thousand fold lacks many of the properties (27) seen with less purified preparations.

We also examined the antiproliferative effects of $rI_n \cdot rC_n$ and its mismatched congener $rI_n \cdot r(C_{13},U)_n$ on human fibroblasts grown in monolayers. Cell plates were treated every two days with fresh polymer duplex at 10^{-4} or 10^{-5} M. After 48 hr, the number of dividing cells began to fall, following exposure to the higher concentration of $rI_n \cdot C_n$; at a lower dose, the $rI_n \cdot rC_n$ effect was not seen until much later, suggesting that a "build-up" of residual polymer may account for this effect. Cells treated with an identical dose of $rI_n \cdot r(C_{13},U)_n$ required a longer time (hence more exposures to the cells) to achieve any measurable effect on cell growth.

The current studies extend our earlier efforts to delineate in various animal systems the therapeutic ratio of mismatched interferon inducers. Collectively, these reports encourage the clinical application of mismatched inducers; accordingly, we are presently engaged in safety testing of a large lot of $rI_n \cdot r(C_{12},U)_n$. It will be of immediate interest to monitor which molecular forms of human interferon are generated, and which secondary biological effects are encountered, when the induction process is triggered by the very brief signal of a mismatched polynucleotide inducer.

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Dr. Leonard Schechtman carried out the experiments concerned with cell growth of the fibroblast cultures.

[3] Leong, S. S., J. S. Horoszewicz and W. A. Carter, unpublished observations.

REFERENCES

1. Field, A. K., Tytell, A. A., Lampson, G. P. & Hilleman, M. R. (1967) *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 1004–1010.
2. Carter, W. A. & De Clercq, E. (1974) *Science* **186**, 1172–1178.
3. Youngner, J. S. & Hallum, J. V. (1968) *Virology*, **35**, 177–179.
4. Freeman, A. I., Al-Bussam, N., O'Malley, J. A., Stutzman, L., Bjornsson, S. & Carter, W. A. (1977) *J. Med. Virol.*, **1**, 79–93.
5. Cornell, C. J., Jr., Smith, K. A., Cornwell, G. G., III, Burke, G. P. & McIntyre, O. R. (1976) *J. Natl. Cancer Inst.*, **57**, 1211–1216.
6. Carter, W. A., Pitha, P. M., Marshall, L. W., Tazawa, I., Tazawa, S. & Ts'o, P. O. P. (1972) *J. Mol. Biol.*, **70**, 567–587.
7. O'Malley, J. A., Ho, Y. K., Chakrabarti, P., Di Berardino, L., Chandra, P., Orinda, D. A. O., Byrd, D. M., Bardos, T. J. & Carter, W. A. (1975) *Mol. Pharmacol.*, **11**, 61–69.
8. Ts'o, P. O. P., Alderfer, J. L., Levy, J., Marshall, L. W., O'Malley, J. A., Horoszewicz, J. S. & Carter, W. A. (1976) *Mol. Pharmacol.*, **12**, 299–312.
9. Carter, W. A., O'Malley, J., Beeson, M., Cunnington, P., Kelvin, A. & Vere-Hodge, A. (1976) *Mol. Pharmacol.*, **12**, 440–453.
10. Pidot, A. L. R., O'Keefe, G., III, McManus, N. & McIntyre, O. R. (1972) *Proc. Soc. Exp. Biol. Med.*, **140**, 1263–1269.
11. Finter, N. B. (1969) *J. Gen. Virol.*, **5**, 419–427.
12. Horoszewicz, J. S., Byrd, D. M., Sokal, J. E. & Carter, W. A. (1974) *J. Natl. Cancer Inst.*, **52**, 649–652.
13. Horoszewicz, J. S. & Carter, W. A. (1974) *Antimicrob. Agents Chemother.*, **5**, 196–198.
14. Carter, W. A., Hande, K. R., Essien, B., Prochownik, E. & Kaback, M. (1971) *Infec. Immun.*, **3**, 671–677.
15. Carter, W. A., Panagos, G. E., O'Malley, J. A. & Freeman, A. I. (1977) *Clin. Res.* **25**, 488A, April.
16. Wheelock, E. F. (1966) *J. Bact.* **92**, 1415–1421.
17. Greenberg, H. B., Pollard, R. B., Lutwick, L. I., Gregory, P. B., Robinson, W. S. & Merigan, T. C. (1976) *New Eng. J. Med.*, **295**, 517–522.
18. Nissen, C., Speck, B., Emödi, G. & Iscove, N. N. (1977) *Lancet*, January 203–204.
19. Ho, M. (1976) *New Engl. J. Med.*, **295**, 562–563.
20. Dean, J. H., Wallen, W. C. & Lucas, D. O. (1972) *Nature New Biol.*, **237**, 218–219.
21. Badger, A. M., Cooperband, S. R. & Green, J. A. (1972) *J. Natl. Cancer Inst.*, **49**, 613–621.
22. Buffett, R. F., Ito, M., Cairo, A. M. & Carter, W. A. (1978) *J. Natl. Cancer Inst.*, **60**, 243–246.
23. Jankowski, W. J., Davey, M. W., O'Malley, J. A., Sulkowski, E. & Carter, W. A. (1975) *J. Virol.*, **16**, 1124–1130.
24. Steinberg, A. D., Baron, S. & Talal, N. (1969) *Proc. Natl. Acad. Sci. U.S.A.*, **63**, 1102–1107.
25. Strander, H., Cantell, K., Carlström, G. & Jakobsson, P. Å. (1973) *J. Natl. Cancer Inst.*, **51**, 733–742.
26. Horoszewicz, J. S., Leong, S. S., Ito, M. Di Berardino, L. A. & Carter, W. A. (1978) *Infec. Immun.*, **19**, 720–726.
27. Carter, W. A., Leong, S. S. & Horoszewicz, J. S. (1978). In "Antiviral Mechanisms and the Control of Neoplasia." (P. Chandra, ed.). Plenum Press, in press.