Journal of Cellular Biochemistry 46:9–20 (1991)

Cellular Response to Double-Stranded RNA

Dale S. Haines, Kenneth I. Strauss, and David H. Gillespie

Department of Neoplastic Diseases, Hahnemann University, Philadelphia, Pennsylvania 19102

Abstract The study of double-stranded RNA (dsRNA) encompasses a variety of fields. Basic research in this area has contributed to a greater mechanistic understanding of gene induction, tumor cell growth arrest, the establishment of antiviral states, and immunomodulation. Because of the possible clinical value of these molecules, physicians are now exploring the use of synthetic dsRNA to treat patients with cancer, HIV-1 disease, and immune dysfunction. Continued studies of the mechanisms of action of dsRNA are likely to suggest an even wider scope of clinical applications.

Key words: polyinosinic acid: polycytidylic acid, antiviral, antiproliferation, gene induction, immunomodulation

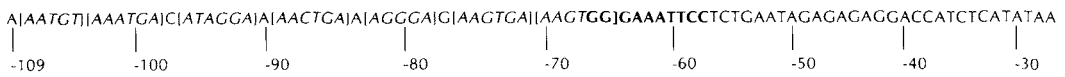
In 1969 Hilleman's group discovered that the interferon inducing component in extracts of *Penicillium funiculosum* was dsRNA [1] and that other natural and synthetic dsRNAs could induce interferon (IFN) formation and host resistance to viral infections in animals and cultured cells [2]. Polyinosinic:polycytidylic acid (pI:C) was the most potent inducer of the RNAs tested, while DNA and single-stranded RNA were consistently inactive. From this basic work, IFN investigators hypothesized that the IFN inducer in virus infected cells was the viral dsRNA genome or replicative intermediate. Subsequently, dsRNA has been found to have several apparently distinct activities. pI:C has been shown to induce a wide variety of genes, activate latent enzymes, arrest tumor cell growth, and enhance both cell mediated cytotoxicity and B cell response to antigens. Although some of these activities follow IFN induction, others are a direct result of dsRNA.

Abbreviations used: AIDS, acquired immunodeficiency syndrome; CHX, cyclohexamide; DDPK, dsRNA dependent protein kinase; dsRNA, double-stranded RNA; eIF-2, eukaryotic initiation factor 2; IFN, interferon; LGL, large granular lymphocytes; NDV, new castle disease virus; NK cells, natural killer cells; PDGF, platelet derived growth factor; pI:C, polyinosinic:polycytidylic acid; PMBC, peripheral blood mononuclear cells; 2AP, 2-aminopurine; 2,5A synthetase, 2′-5′ oligoadenylate synthetase; 2′-5′A, 2′-5′ oligoadenylates; VSV, vesicular stomatitis virus.

Received November 16, 1990; accepted January 3, 1991.

GENE INDUCTION BY dsRNA OR VIRUS INFECTION

The molecular mechanisms which contribute to the induction of genes by dsRNA or virus infection are best known in the case of Type I IFNs (α and β). It has been well established that these genes are transcriptionally regulated by dsRNA or virus. Researchers have defined cis-acting regulatory and promotor (Fig. 1) regions of the IFN-β gene which are required for induction by pI:C or virus [reviewed in 3, 4]. A number of IFN regulatory factors (IRF) which bind to sequences within the human IFN-β promotor region have been identified. These include IRF-1, IRF-2, and NF-kB [reviewed in 3]. Both IRF-1 [5], involved in enhancing transcription, and IRF-2 [6], involved in the down regulation of transcription, bind to the same hexamer repeat within the promotor region of the human IFN-β gene. IRF-1 may also play a role in the induction of IFN-α since a transfected murine IRF-1 gene was capable of inducing transcription of both α and β IFN in uninduced monkey COS cells [7]. Along with IRF-1 and IRF-2, Visvanathan and Goodbourn [8] recently identified a cellular factor in L929 and C127, activated by pI:C, that binds to another sequence (−65 to −55) within the IFN-β promotor and enhances transcription. This inducible factor has DNA binding specificities indistinguishable from transcription factor NF-kB previously identified in mature B cells. Lenardo et al. [9] later demonstrated that



IRF-1 and IRF-2 binding sites are in italics; **NF-kB binding sites are in bold type.**

Fig. 1. Promotor region of the human IFN-β gene [4].

the NF-kB and IFN-β regulatory sequences (−65 to −55) were interchangeable in vivo and that viral treatment of 70Z/3 pre-B lymphocytes induced κ gene expression as well as β IFN gene expression. Discrepancies have arisen in establishing the importance of transcription factors *[10] and cis-acting* sequences [11,12,13] involved in the regulation of IFN-β gene expression. These discrepancies may be explained by the use of transfected versus resident genes [10], or the differential expression, utilization, and modification of transcription factors present in the different cell lines studied. Goldfield and Maniatis [14] first developed this idea after finding that cyclohexamide (CHX) had no effect on the induction of either tumor necrosis factor α (TNF-α) or IFN-β in Sendai virus infected U937 monocytoid cells, while CHX treatment did block induction of both genes in the Namalwa B cell line and IFN-β in the JY B cell line. They concluded that cell lines differ in their ability to constitutively produce the positive and negative regulatory proteins required for IFN-β induction by dsRNA or viral infection. This view, echoed also by Taylor and Grossberg [3], appears to be a powerful framework from which to understand the biological activity of dsRNA and other cytokines which have pleiotropic activities.

It is less well understood how dsRNA regulates the induction of other genes involved in the antiviral or growth mediated response. The known genes directly induced by pI:C are listed in Table I. In this review, *direct induction* is defined as specific RNA accumulation in the presence of the protein synthesis inhibitor CHX. This accumulation must be greater than in the presence of CHX alone, since previous studies have noted induction of dsRNA inducible genes with CHX [129]. Experiments have also shown induction of other genes by dsRNA or virus *(Table II)* without CHX. Although several homologies have been claimed among promoter regions of dsRNA inducible genes as illustrated above, they exist in positions or orientations different from the IFN-β regulating elements. More work is needed to establish whether the various dsRNA inducible genes and IFN-β are regulated by similar or unrelated transcription factors.

The mechanism by which dsRNA leads to the activation of certain cellular factors and subsequent gene induction remains unclear. Since protein synthesis is apparently not required for the induction of some genes, activation of transcription may be the consequence of post-transcriptional modification of preexisting regulatory proteins [15]. Also, since a single molecule of dsRNA is sufficient for the induction of type 1 IFN, Marcus and Sekellick [16] proposed that dsRNA triggers amplification of a cellular signal. It is possible that this dsRNA triggered signal may be a result of activated protein kinases capable of regulating a wide range of factors involved in the regulation of dsRNA inducible genes. Zinn et al. [17] showed that 2-aminopurine (2AP) inhibited the induction of *c-myc, c-fos,* and IFN-β in the pI:C treated human MG63 cell line. Marcus and Sekellick [18] showed that 2AP inhibited the induction of type 1 IFN in vesicular stomatitis virus (VSV) treated mouse L(Y) and chick embryo cells. Since dsRNA dependent protein kinase (DDPK) and the heme regulated eIF-2 kinase are the only known protein kinases which are inhibited by 2AP, both groups proposed the possibility that DDPK may play a role in dsRNA regulation of cellular proteins. Ghosh and Baltimore [19] demonstrated

TABLE I. Known Genes Directly Induced by dsRNA or Virus (in the presence of CHX)

Gene	Cell line	Inducer	Ref
IFI-78k	Human diploid fibroblast	NDV, pI:C	122
IFI-56k	HeLa, UAC, FS-4, Daudi, Vero	pI:C	123
IFI-54k	Daudi, DIF-8	NDV, pI:C	124
c-myc	Balbc/3T3	pI:C	125
c-fos	Balbc/3T3	pI:C	125
JE	Balbc/3T3	pI:C	125
IRF-1	Hela	pI:C	10
IFN-β	Balbc/3T3	pI:C	125

TABLE II. Other Genes Which May Be Directly Induced by dsRNA (no CHX data)

Gene	Cell line	Inducer	Ref
TNF-α	U937	Sendai	14
KC	Balbc/3T3	pI:C	124
MCSF	Balbc/3T3	pI:C	124
IL-6	Hela	pI:C, Sendai	125
IFN-α genes	Namalwa	Sendai	126
TIS7	Swiss 3T3	NDV, pI:C	127

that phosphorylation of the NF-kB inhibitor, IFB, by protein kinase C or the heme regulated eIF-2 kinase led to IFB inactivation and subsequent activation of NF-kB, while the cyclic AMP dependent protein kinase had no effect. Since DDPK has similar substrate specificity as the heme regulated protein kinase, future experiments should investigate whether DDPK is capable of inactivating IFB. Experiments should also investigate the ability of DDPK to activate other protein kinases. A full knowledge of the basal activity of relevant regulatory and signal transduction systems will be essential in revealing the mechanisms of gene induction by dsRNA in any biological setting.

dsRNA DEPENDENT ACTIVITIES IN CELLS

Early in the 1970's it was discovered that dsRNAs of natural or synthetic origin inhibited viral RNA and protein synthesis in IFN treated intracellular systems [reviewed in 20]. It was later shown that two dsRNA dependent enzymes, 2,5A synthetase and DDPK, contributed to this inhibition. Upon activation by dsRNA, 2,5A synthetase converts ATP into a thermostable compound, 2,5A. This compound then activates a latent endonuclease, RNase L, which subsequently degrades host and viral RNA. Several forms of the 2,5A synthetases, with different dsRNA requirements and subcellular localizations, have been isolated from individual cell types [21,22]. Optimal activity for the 30 to 40 kD synthetase requires 100 to 1,000 times more dsRNA than the 110 kD isozyme. Recently, Suhadolnik et al. [23] also showed that fructose 1,6-bisphosphate activated the 110 kD synthetase purified from both HeLa cells and rabbit reticulocyte lysate. It remains to be seen, however, which other isozymes can be activated by this cellular metabolite. Other intermediates of glycolysis or other phosphorylated sugars did not display this activity. The preparations of sugar were shown to be free of dsRNA by nuclease resistance. In light of the fact that approximately 70 bp of dsRNA are required to activate 2,5A synthetase [24], it is curious that a phosphorylated sugar monomer is also active. The role of a glycolytic intermediate in activating the 2,5A synthetase/RNase L pathway may have repercussions for regulation of cell cycle metabolism.

The basis for specificity of RNA degradation by the 2,5A synthetase/RNase L pathway, if such specificity exists, remains elusive. Activation of RNase L at the site of viral replication might facilitate preferential degradation of foreign RNA. The 2,5A synthetases may act locally on RNase L, making the subcellular site of the enzyme pathway important. The 110 kD isozyme, which requires low amounts of dsRNA for optimal activation (0.1 μg/ml), might be responsible for selective translational control; the 67 kD membrane associated synthetase for signal transduction, the 30 to 40 kD nuclear isozyme for hnRNA cleavage [25]. The differential expression and localization of the synthetase isozymes makes them candidates in the mechanisms of cell cycle regulation and differentiation.

Along with 2,5A synthetase, DDPK has also been shown to inhibit protein synthesis in IFN treated cells [reviewed in 20, 26]. Activation of DDPK requires dsRNA binding, ATP binding in the presence of manganese, and autophosphorylation. Once activated, DDPK phosphorylates the 37 KD eukaryotic initiation factor subunit, eIF-2α [27], and certain histones [28,26]. Phosphorylated eIF-2α binds the guanine nucleotide exchange factor, eIF-2B, and subsequently prevents further initiation of protein synthesis [29,30]. Galabru et al. [31] recently purified the 68 kD DDPK from Daudi cells utilizing monoclonal antibody affinity chromatography and demonstrated by Scatchard analysis that the protein had both high and low affinity binding sites for pI:C. These results concur with previous work which established that optimal concentrations of dsRNA activated kinase activity, while high concentrations inhibited DDPK activity [32]. Kostura and Mathews [33] also performed Scatchard analyses using purified DDPK activated with heteropolymer dsRNA and discovered only one binding site. They also found that activation was second order with respect to DDPK concentration, suggesting that activation

of DDPK required one dsRNA molecule and two DDPK molecules. Also suggestive of an intermolecular catalytic mechanism, histones that bound dsRNA with high affinity relieved the inhibition of DDPK by excessive dsRNA concentrations [34]. DDPK bound to dsRNA could autophosphorylate free DDPK but not other dsRNA-bound enzyme molecules. Further work is needed to resolve conflicting binding site results and to establish the exact mechanism by which dsRNA *activates DDPK.*

dsRNA may also inhibit protein synthesis by other mechanisms. Kaempfer and his colleagues [35] discovered that dsRNA can bind to and inactivate eukaryotic initiation factor-2 (eIF-2) and subsequently inhibit protein synthesis. Harary et al. [36] learned that 2 μg/ml of pI:C, which was coadministered with DEAE dextran in order to promote dsRNA uptake, caused a generalized inhibition of protein synthesis in L929 cell, while 20 μg/ml of pI:C administered alone did not. A cytopathic effect could not be demonstrated in any cultures by vital dye staining. While general protein synthesis was inhibited by over 80%, IFN activity (antiviral) was markedly induced. These experiments provided evidence that high intracellular concentrations of pI:C can cause a more or less generalized inhibition of protein synthesis, but additional factors must be required for achieving cell lysis. Harary et al. [36] then found that when an eIF-2:pI:C complex was applied to cells in the presence of DEAE dextran, IFN induction by pI:C in cells and antiviral activity of pI:C in mice were augmented while protein synthesis inhibition was reduced. It remains to be established whether coupling of dsRNA with eIF-2 prevented dsRNA uptake or spared the intracellular eIF-2 pool in releasing protein synthesis inhibition. Nevertheless, these results suggest that dsRNA may inhibit protein synthesis by direct interaction with eIF-2, in addition to working through DDPK and 2-5A synthetase.

Along with impairing virus replication through the inhibition of protein synthesis, activation of 2,5A synthetase/RNase L pathway and DDPK, recent work has demonstrated the ability of dsRNA to induce other activities that may be related to dsRNA's antiviral effects. Meegan and Marcus [37] demonstrated that dsRNA induced in avian cells a secreted soluble nuclease that *degrades dsRNA.* They also noted that under different assay conditions, mammalian cells also released and accumulated high activities of dsRNase [37]. Also, an activity that unwinds dsRNA has been reported to exist in several organisms. This activity was first shown in *Xenopus laevis* oocytes wherein injected antisense RNA was unable to form stable hybrids in vivo with its complementary endogenous mRNA [38]. Bass and Weintraub [39] later showed that this unwinding activity covalently modified dsRNA by converting adenosine residues to inosine residues. This conversion altered the base pairing of RNA duplexes, rendering them susceptible to single-stranded RNases [39]. Wagner and Nishikura [40] have also shown this activity in various types of mammalian cells including HeLa, human lymphoblastoid, mouse plasmacytoma, Burkitt lymphoma, and mouse F9 teratocarcinoma cells. They also demonstrated a saturable, reversible binding of proteins to dsRNA by gel retardation assays [40]. The role of dsRNA unwinding activity has been implicated in cell cycle control and differentiation [38]. It is also possible that the unwinding activity is part of another cellular defense mechanism against viruses.

ANTIVIRAL ACTIVITY OF dsRNA AND ITS ROLE IN THE REGULATION OF HIV-1 GENE EXPRESSION

It is generally thought that all viruses elaborate dsRNA intermediates during infection and that the presence of these intermediates affords the cell some level of resistance to infection via IFN induction, activation of dsRNA-dependent enzymes, and immune inhancement. When exogenously applied prior to virus infection, both natural and synthetic dsRNAs exhibited antiviral activity in vivo and in vitro [1,2,41,42]. Activation of the 2,5A synthetase/RNase L pathway by dsRNA may account for protection against some viruses. Chebath et al. [43] found that CHO cells expressing a transfected 100 kD 2,5A synthetase gene exhibited elevated 2,5-A levels, and were less susceptible to certain viral infections. In addition, inhibition of the antiviral state generated by microinjection of RNase L-activating 2,5A and 2,5A analogs was demonstrated by coinjection of the S_pS_p stereoisomer of 2,5A, a specific inhibitor of RNase L [44].

Recently, the so-called Mx proteins have been shown to protect mice from influenza virus infection [45]. Even though various analogues of these proteins have been identified in a variety

of animal species [46,47,48], their antiviral activity has been verified only in mice. VSV alone did not induce Mx (p78); however, pretreatment of cells with IFN-γ resulted in augmented production of Mx transcripts upon subsequent exposure to dsRNA or virus [49]. Neither 2,5A synthetase nor Mx alone protected against VSV infection. Goetschy et al. [49] hypothesized that IFN priming allows cells to accumulate antiviral molecules, which are promptly activated upon viral infection and subsequently inhibit protein synthesis in infected cells and delay virus multiplication. However, in unprimed cells, these molecules might be induced by viruses, but the kinetics of viral takeover would interfere with the establishment of an antiviral state.

Some viruses have evolved mechanisms which confer resistance to the antiviral effects of dsRNA or IFN [reviewed in 4]. This may arise from virus mediated inhibition of the DDPK and 2,5A synthetase/RNase L pathways. For example, the accumulation of non-functional 2-5A and consequent elimination of RNase L activity occurs in herpes simplex I, II [50], SV40 [51], and vaccinia virus [130] infected cells. Also, reovirus encodes for a well characterized dsRNA binding protein, reovirus σ3 [52], which inhibits DDPK activation in infected L929 cells [53]. Resistance to IFN by adenovirus has been attributed to VAI RNA which competitively binds to DDPK and prevents its activation by dsRNA [reviewed in 54, 55].

Human immunodeficiency virus type-1 (HIV-1) may also be relatively resistant to IFN and dsRNA mediated antiviral effects and may also use genomic dsRNA regions to up-regulate its own gene expression. Replication of HIV-1 requires the viral encoded TAT protein [56]. Activation of HIV-1 transcription by TAT is mediated via TAR sequences present in the 5′ region of the LTR [57]. These sequences possess a high degree of secondary structure (Fig. 2) and are present in all HIV-1 transcripts [58]. Subsequent studies have shown that these sequences were capable of inhibiting translation of downstream sequences. Parkin et al. [59] demonstrated that synthetic RNA derived from sequences +1 to +111 of the LTR were capable of inhibiting translation of a downstream linked report gene in reticulocyte lysates, HeLa cell extracts, and *Xenopus laevis* oocytes. This inhibition was eliminated by mutations which disrupted the predicted secondary structure. Compensation mutations which once again restored the secondary structure resulted in the renewed inhibition of protein synthesis. They suggested that the 5′-secondary structure interfered with translational factor binding at the CAP site and subsequent protein synthesis.

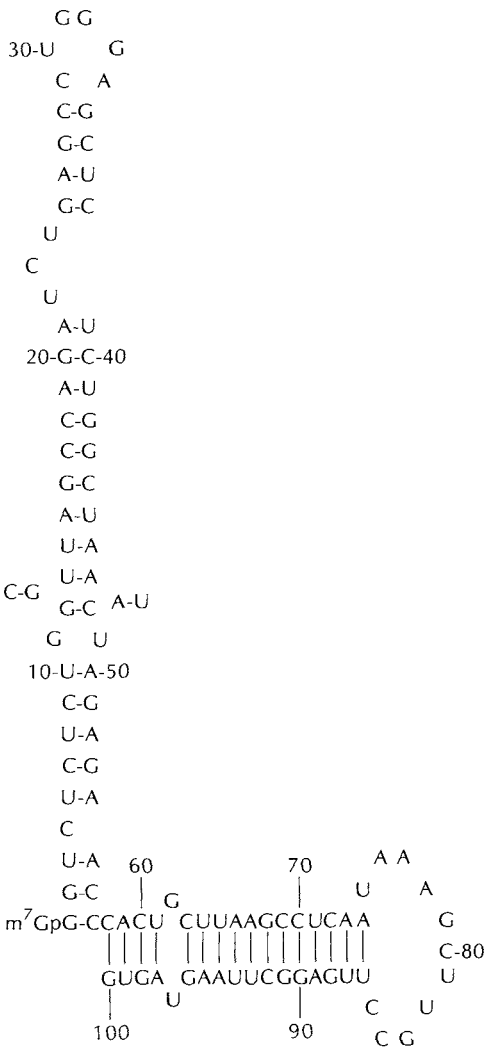


Fig. 2. Secondary structure proposed for HIV-1 leader mRNA [128].

It has also been proposed that mRNA encoded TAR sequences can inhibit protein synthesis in trans. This is supported by previous work which has shown that synthetic RNAs transcribed from cloned TAR sequences were capable of activating DDPK and promoting subsequent phosphorylation of eIF-2 (+1 to +80 [60], and +1 to +232 [61]). SenGupta and Silverman [61] showed activation of 2,5A synthetase by these same synthetic RNAs (+1 to +111). HIV mediated inhibition of translation has been linked to

the long latency between HIV-1 infection and the development of acquired immunodeficiency syndrome (AIDS). Roy et al. [62] hypothesized that HIV may be induced from a latent state by an increase in TAT levels, since TAT was capable of down-regulating DDPK protein levels in IFN treated HeLa cell stably expressing TAT. They also stated that this down-regulation was specific for DDPK since there was no decrease in 2,5A synthetase message in IFN treated HeLa cells. However, Schröder et al. [63] demonstrated that TAT was capable of binding to the 5′-leader RNA and subsequently inhibited its ability to bind and activate purified 2,5A synthetase. In contrast to the previous mentioned reports, Gunnery et al. [64] demonstrated that synthetic TAR RNA (+3 to +82) purified to apparent homogeneity was unable to activate purified DDPK. At high concentrations, however, this RNA prevented activation by reovirus dsRNA. Gunnery et al. [64] concluded that results of previous work documenting activation of DDPK by these same sequences may be due to contaminating dsRNA molecules generated from the in vitro transcription system utilized and not by actual TAR RNA. It is also possible that nucleotides +83 to +111, of the Gunnery et al. [64] transcripts' are required for DDPK activation. Nonetheless, inhibition of the two dsRNA dependent intracellular enzymes by HIV-1 mRNA and TAT may aid its ability to avoid host cellular defense mechanisms. Along with the inhibition of these enzymes, HIV-1 leader mRNA may also be involved in the induction of HIV-1 gene expression. The activation of NF-kB may be a common pathway in increasing HIV-1 expression and activation of latent provirus, since mutations within sequences of the LTR enhancer region (sequences similar to the k light chain enhancers) prevented both binding of NF-kB and subsequent HIV-1 transcription [131]. Visvanathan and Goodbourn [8] demonstrated that pI:C was capable of activating the binding of NF-kB to the inducible element within the human β IFN promoter and activate transcription. It is therefore possible that the double-stranded region of leader sequences or other dsRNA replicate intermediates of HIV-1 may be able to activate HIV-1 expression through NF-kB.

The activation of HIV-1 expression by dsRNA notwithstanding, Montefiori and Mitchell [65] showed that the HIV-1 permissive cell lines, C3 and CEM, were significantly protected from HIV-1 infection when grown in media supplemented with a mismatched analog of pI:C, pI:C,U. pI:C,U inhibited proviral DNA synthesis and RNA accumulation in virus challenged T-lymphoblastoid cells [66]. A single exposure of pI:C,U had greater antiviral effects than a single exposure IFN-α and IFN-β on CEM cells or combined exposure of these cytokines on C3 cells [65]. dsRNA showed synergy between IFNs and other antiviral compounds with pI:C,U in inhibiting HIV-1 infection [67]. However, in the same study, pI:C,U was unable to inhibit the production of virus from chronically infected cell lines. pI:C,U was effective against a genetically divergent isolate of HIV-1 (HTLV-III_{RF}) and possessed antiviral activity in non-$CD4^+$ cell lines of monocyte/macrophage (U937) origin [132]. Lawrence et al. [68] also demonstrated a translational block of HIV-1 replication in acutely infected cells and confirmed the lack of antiviral activity of pI:C,U in chronically infected cell lines.

In vitro, HIV-1 infection of at least some lines of $CD4^+$ T cells results in a temporary elevation of 2-5A synthetase and RNase L activities; however, these activities decline as HIV-1 infection persists [69]. Previous studies have established the importance of these enzymes in mediating resistance to HIV-1 infection. High levels of 2,5 A synthetase, supplied by transfected 2,5A synthetase genes, prolongs suppression of HIV-1 infection [70]. Also, RNase L association with the nuclear matrix may degrade HIV *env* RNA [69]. The regulation of HIV-1 infection by dsRNA activated enzymes and vice versa is obviously complex. Considering that dsRNA protects $CD4^+$ and macrophage cells from HIV-1 infection and has immunomodulatory action, it may be useful to consider combination therapy with reverse transcriptase inhibitors (AZT, ddI). Such a combination may also reduce the frequency of AZT resistant viruses and prolong the T4 cell increases which are transiently obtained with AZT (W.A. Carter and D. Strayer, personal communication).

Antiproliferation Activity of dsRNA

Levy et al. [71] were the first to show that pI:C could inhibit the rate of growth of human tumor xenografts in mice. Fisher et al. [72] provided evidence that immune enhancement alone could not explain the antitumor activity of pI:C and forecasted that another mechanism must also be involved. Levy [73] discovered that

20–100 μg/ml of pI:C was "toxic" to a variety of proliferating cells. Initially, it was not recognized that the cytostatic (antiproliferative) and cytotoxic responses to pI:C were distinct phenomena. Subsequent research showed that low concentrations (e.g., 1 μg/ml) of dsRNA selectively inhibited tumor cell growth [74,75,76]. This inhibition of cell growth could be explained by IFN induction in some cell lines [77,78], while in other cell lines, sensitivity to dsRNA and IFN were often independent phenomena [79,80]. It has been generally assumed that the IFN independent antiproliferative activity of dsRNA involves activation of the dsRNA-dependent intracellular enzymes, 2-5A synthetase and/or DDPK. Chapekar et al. [78] showed induction of 2,5A synthetase and degradation of rRNA after dsRNA application. Although greater than 10 μg/ml of pI:C was required to detectably increase 2-5A synthetase, 0.5 μg/ml inhibited DNA synthesis and cellular proliferation. Arad et al. [81] reported that antiproliferation by dsRNA occurred most dramatically in cells with high 2-5A synthetase levels. In neither case was it possible to obtain evidence that DDPK might be involved.

Two new discoveries raise an interesting, new possibility. Suhadolnik et al. [23] showed that 2,5A synthetase can be activated in vitro by 1–10 mM fructose 1,6-bisphosphate to produce bioactive 2,5A. Hubbell et al. [82] have discovered that adenyl cyclase becomes activated in dsRNA treated cells and that cAMP increases are necessary and sufficient for the antiproliferative activity of pI:C and pI:C,U. These results suggest that dsRNA may exert its antiproliferative effect through cAMP signal transduction, activating glycogen breakdown and subsequent increases in fructose 1,6-bisphosphate concentrations [23,83]. It should be pointed out, however, that the antiproliferative activity of IFN is probably not exerted through this route, since application of IFN to the same tumor cell lines does not result in cAMP accumulation. Whether dsRNA effects synergy with IFNs through adenyl cyclase activation needs clarification.

dsRNA has been shown to have differential effects on fibroblasts. Forsberg et al. [74] reported that 2 μg/ml of pI:C inhibited the platelet derived growth factor (PDGF) stimulated growth of human foreskin fibroblasts rendered "quiescent" by 2 days of culture in serum-free medium. Treatment with pI:C after initiation of DNA synthesis (about 16 h after PDGF addition) did not have any inhibitory effect on proliferation. pI:C (10 μg/ml) also inhibited [^{3}H] thymidine incorporation in response to other growth factors (FGF, 10 ng/ml and EGF, 2 ng/ml). Neither protein synthesis nor the interaction between PDGF and its receptor were disturbed by pI:C treatment. These results are consistent with a mechanism of autogenous production of IFN-β_2 in response to pI:C. Zullo et al. [75] showed that pI:C applied to mouse BalbC/3T3 fibroblasts, rendered "resting" by growing to confluence in 5% calf serum, mimicked PDGF in causing a proliferative response subsequent to induction of competence genes. Vilcek et al. [76] then demonstrated a mitogenic response in cycling human foreskin fibroblasts and showed that the proliferative response was intensified if IFN-β was neutralized with antibodies after pI:C application, developing the idea that IFN may naturally feed back to limit proliferation subsequent to mitogenic signals. Growth and antigrowth signals transmitted by pI:C or pI:C,U are multiple, complex, and poorly understood. The multiplicity of responses may derive from the complex structure of the homopolymer dsRNAs which are comprised of both heterogeneously sized single-stranded and double-stranded domains. A fruitful area of future research may be the study of dsRNA of defined structure, which may aid in determining whether certain structures elicit only one or a few of the many possible growth responses.

IMMUNOMODULATION

In vivo administration of dsRNA has been reported to enhance cytolytic activity of natural killer (NK) cells [84,85] and monocytes [86] and to increase humoral immunity [87]. In no case has a cell directly responsive to dsRNA been unambiguously identified. It is therefore possible that the observed immune enhancements are secondary to response of unidentified accessory cells. Immune enhancement occurs through the increased recruitment of precursor cells through IFN production. In Ficoll-purified blood mononuclear cells, dsRNA induced the secretion of IFN-α, -β and -γ [88]. However, neither lymphocytes (nonadherent) nor monocytes (adherent) separated from peripheral blood mononuclear cells (PBMC) could secrete IFN-α [88]. dsRNA can cause accumulation of certain mRNAs in PBMC, such as the mRNA coding for TNF-α and TNF-β [89]. Purified subpopulations of immune cells and cell lines resembling

specific types of immune cells can also respond to dsRNA by synthesizing lymphokines, but often the relationship between cytokine expression and immune enhancement is unclear.

Monocytes/Macrophages

Macrophage activation, the production of macrophages capable of lysing susceptible target cells, is thought to involve two distinct steps: priming and triggering. Though pI:C can activate responsive macrophages directly, this is also considered to be a two-step process [90]. Target cell lysis by dsRNA activated macrophages can occur via release of TNF-α [91] or by TNF-α + IFN-α/β [92] from macrophages [133]. Fleit and Rabinovitch [93] showed release of type I IFN (α/β) by mouse bone marrow macrophages treated with pI:C or infected with new castle disease virus (NDV). In the human monocytoid cell line, U937, Sendai virus infection resulted in increased rates of TNF-α and IFN-β mRNA synthesis [14]. This mRNA synthesis was partially sensitive to 10 mM 2-AP but resistant to 50 μg/ml of CHX, suggesting that activation of DDPK was required but new protein synthesis was not. In contrast, TNF-α and IFN-β mRNA synthesis was sensitive to 2AP and CHX in Namalwa and JY human B cell lines [14]. Whether Sendai virus is mimicked by pI:C in U937 cells, and whether similar mRNA induction occurs in natural macrophages needs further clarification.

Lepe-Zuniga et al. [88] reported that while pI:C and pI:C,U induced increases in IFN-β activity in purified human monocytes/macrophages, unless IFN-γ was also added, no IFN-α activity was produced. This finding suggested that dsRNA induction of IFN-α in macrophages required IFN-γ production by accessory cells such as lymphocytes. Once macrophages were primed by exogenous IFN-γ, enhanced tumoricidal activity followed low dose application of pI:C [94]. It is curious that though macrophage activation is a two-step process, the direct induction of two molecules thought to be utilized in the terminal cytolytic event, TNF-α and IFN-β, are probably directly induced during priming with pI:C [95]. Triggering may involve either superinduction of these proteins and/or induction of other proteins used for binding to and/or lysis of target cells, such as complement proteins [96]. It is possible that the reason that high concentrations of pI:C directly activate responsive macrophages is that enough dsRNA remains after priming to trigger the primed cells. It would be interesting to learn whether two low doses of pI:C separated in time would be sufficient to activate responsive macrophages. It is not known whether by activating macrophages pI:C mimics a natural dsRNA [97] or a natural non-dsRNA [20].

NK Cells and T Cells

pI:C and pI:C,U increased NK activity in vivo in animals [84], man [98], and in vitro [99]. In vivo increases in cytolytic activity, following a single IV injection of pI:C, coincided with increases in the number of circulating NK cells. This increase in NK cell number could not be explained by proliferation of NK cells, but probably arose through recruitment of NK cells from other localities [100,101]. dsRNA does not directly activate T cells in vitro. However, LAK T cell cytolytic activity was induced over 3–4 days by pI:C,U in combination with doses of IL-2 which were in themselves too low to induce LAK cytolytic activity [H.R. Hubbell and R. Bigler, personal communication].

Because of the difficulty in purification of NK cells, little is known about the mechanism of NK enhancement by dsRNA. Increase in NK cytotoxic activity in vitro by pI:C and pI:C,U was first discovered by exposing Ficoll-purified blood mononuclear cells to dsRNA for 24 h and measuring killing of the NK target cell line, K562 [99]. It is possible that enhancement of NK activity by dsRNA required accessory cells since no published experiments examine activation of purified large granular lymphocytes (LGL) by dsRNA. Indeed, Cohen et al. [102] reported that macrophages may release IFN-α and IFN-β and thereby activate NK cells. Santoni et al. [103] showed that in vivo administration of pI:C resulted in increased fibronectin synthesis by NK cells and found that fibronectin was required for post-binding induction of phosphatidyl inositol hydrolysis. A population of cells purified as plastic nonadherent, nylon nonadherent, blood mononuclear cells, containing NK cells and T cells, was shown to synthesize IFN-γ mRNA in response to pI:C [134]. Unlike induction of IFN-α and -β mRNA in fibroblasts and monocytoid cells, induction of IFN-γ mRNA required prior de novo protein synthesis [135] and activation of protein kinase C [104,105].

B Cells

Braun and Nakano discovered that oligodeoxynucleotides [106], poly(A:U) [87], or poly(C) complexed with basic proteins [87] injected into CF-1 or AKR mice 48 h after injection of sheep red cells stimulated increases in the number of spleen cells releasing antibody to sheep red cells. Later work showed that pI:C [107] and mycovirus dsRNA [108] produced similar effects. The stimulation only occurred when RNA was administered *after* antigen; otherwise *suppression* of humoral immunity resulted. More recent research suggests that the suppression may have occurred secondary to NK cell activation [109].

Goldfield and Maniatis [14] demonstrated protein synthesis independent induction of IFN-β and TNF-α mRNA by Sendai virus infection in human B cell lines. Earlier, Shuttleworth et al. [110] had shown coordinate induction of mRNA coding for IFN-α and IFN-β in Namalwa B cells by Sendai virus infection, but it was not determined whether prior protein synthesis was necessary. Thus, it is possible that B cell enhancement in vivo is a result of dsRNA's direct effects on other cells.

CLINICAL APPLICATIONS

Because dsRNAs in vivo have antiviral activity, inhibit tumor cell proliferation and enhance immune function, it was natural to develop them as new clinical entities. pA-U, pI:C, and pI:C,U have all been used clinically with reported success. Surprisingly, even though the antiviral properties of dsRNA were discovered first and plausible mechanisms of actions had been established through activation of intracellular dsRNA dependent enzymes, the first clinical tests of dsRNA were as anticancer agents. Regarding pI:C, two opposing strategies were implemented in the clinic. First, it was reasoned that the most powerful clinical activities would result if the dsRNAs were stabilized by complexing with a basic protein. pI:C·LC, pI:C complexed with polylysine and carboxymethyl cellulose, was used in a phase 1 clinical trial to evaluate toxicity [111]. Though some evidence of clinical response was obtained, pI:C·LC was found to be toxic and doses predicted to be therapeutic could not be reached. In the second strategy, it was reasoned that the induction of IFN required only brief cell contact with dsRNA, so the lowest toxicity would result if biodegradation in vivo were accelerated. pI:C,U is rapidly degraded in blood [112], but can induce IFN and exhibits antiviral [65,113], antitumor [114], and NK activating [99] activity.

pI:C,U was shown to be without significant clinical toxicity in cancer patients, even when given twice weekly at relatively high doses (200–1,000 mg) for several years [115,116,114]. Clinical responses were seen at doses above 100 mg, including some complete remissions [116], but the major response observed was disease stabilization. In the few cases examined, tumor regression was accompanied by increases in 2,5A synthetase in PBMC and increases in intracellular bioactive 2,5A [118]. The extent to which cancer responses resulted from direct action on the tumor vs. enhanced immune surveillance has not been determined. More recently, trials with pI:C,U and IFN-α in cancer patients have resulted in an unexpected higher response rate [D.R. Strayer and I. Brodsky, personal communication]. D. Strayer (personal communication) has proposed that the chance of cancer response is linked to direct antitumor actions of dsRNA, while the duration of responses is more related to immune enhancement.

pI:C,U was reported to have beneficial effects in 10 HIV disease patients [119]. T4 cell numbers stabilized, delayed cutaneous hypersensitivity returned, and HIV load slowly decreased. A pilot study of 30 additional patients confirmed these responses [117]. A large trial in HIV disease failed, probably due to misformulation of the dsRNA [117]. The effects in HIV disease may have to do with an intrinsic defect in the 2,5A synthetase pathway in PBMC. It is reported that PBMC of ARC and AIDS patients have elevated levels of latent 2,5A synthetase [119,120,121] and low to undetectable levels of activated RNase L [118,119,121]. After administering pI:C,U for several weeks, these values normalized [119,121]. It is unlikely that the observed 2,5A synthetase/RNase L defect is the disturbance in the pathway resulting from HIV infection which has been observed in vitro [69], because so few PBMC are infected in vivo. Alternatively, the pathway defect may signal a cytokine imbalance which can be corrected by dsRNA. Such an effect may signify that immune enhancement is a more important property in controlling HIV disease than its direct anti-HIV activity.

PERSPECTIVE

Biological responses to exogenously supplied dsRNA are varied and fascinating. Some responses are direct, such as the rapid and specific induction of cAMP in tumor cells leading to growth arrest. Other responses are more complex, such as the immune enhancements which may begin with control of specific (IFN) gene transcription in one cell type and culminate in enhanced immunoactivity of secondary or tertiary affected cells. Still others, such as generation of an antiviral state and toxicity, may result from direct action of dsRNA as an intracellular cofactor for enzyme activity. These responses not only provide fertile ground for scientific inquiry, but they also promise new clinical approaches to diseases involving neoplastic growth, virus infection/reactivation, and/or immune dysfunction.

Nevertheless, many important areas of dsRNA biology remain unclear. Does dsRNA exert antitumor cell activity through cAMP by differential gene regulation, protein phosphorylation, or both? What are the signal transduction pathways leading to direct and indirect gene transcription by dsRNA? What primary-secondary cell interactions occur in vivo to cause immune enhancement by dsRNA? Is there a unitary mechanism for generation of antiviral states? Have these responses to dsRNA evolved because dsRNA itself is an important cellular signal or because it structurally mimics other bioactive molecules? It is hoped future research will answer some of these questions.

ACKNOWLEDGMENTS

We acknowledge the support of grant CA29545.We are grateful to H. Hubbell, D. Strayer, and W. Carter for helpful discussions.

REFERENCES

1. Lampson GP, Tytell AA, Field AK, Nemes MM, Hilleman MR: Proc Natl Acad Sci USA 58:782–789, 1967.
2. Field AK, Tytell AA, Lampson GP, Hilleman MR: Proc Natl Acad Sci USA 58:1004–1010, 1967.
3. Taylor JL, Grossberg SE: Virus Res 15:1–26, 1990.
4. Taniguchi T: J Interferon Res 9:633–640, 1990.
5. Fujita T, Sakakibara J, Sudo Y, Miyamoto M, Kimura Y, Taniguchi T: EMBO J 7:3397–3405, 1988.
6. Harada H, Fujita T, Miyamoto M, Kimura Y, Maruyama M, Furia A, Miyata T, Taniguchi T: Cell 58:729–739, 1989.
7. Fujita T, Kimura Y, Miyamoto M, Barsoumian E, Taniguchi T: Nature 337:270–272, 1989.
8. Visvanathan KV, Goodbourn S: EMBO J 8:1129–1138, 1989.
9. Lenardo MJ, Fan CM, Maniatis T, Baltimore D: Cell 57:289–294, 1989.
10. Pine R, Decker T, Kessler DS, Levy DE, Darnell JE: Mol Cell Bio 10:2448–2457, 1990.
11. Pitha PM, Ciufo DM, Kellum M, Ray NBK, Reyes GR, Hayward GS: Proc Natl Acad Sci USA 79:4337–4341, 1982.
12. Tavernier J, Gheysen D, Duerink F, Van der Hayden J, Fiers W: Nature 301:634–636, 1983.
13. Moroteaux C, Kahana C, Mapory Y, Groner Y, Revel M: EMBO J 2:325–332, 1983.
14. Goldfield AE, Maniatis T: Proc Natl Acad Sci USA 86:1490–1494, 1989.
15. Keller DA, Maniatis T: Proc Natl Acad Sci USA 85: 3309–3313, 1988.
16. Marcus PI, Sekellick MJ: Nature 266:815–819, 1977.
17. Zinn K, Keller A, Whittemore LA, Maniatis T: Science 240:210–213, 1988.
18. Marcus PI, Sekellick MJ: J Gen Virol 69:1637–1645, 1988.
19. Ghosh S, Baltimore D: Nature 344:678–681, 1990.
20. Lengyel P: J Interferon Res 7:511–519, 1987.
21. Ilson DH, Torrence PF, Vilcek J: J Interferon Res 6:5–12, 1986.
22. Chebath J, Benech P, Hovanessian A, Galabru J, Revel M: J Biol Chem 62:3852–3857, 1987.
23. Suhadolnik RJ, Li SW, Sobol RW, Varnum JM: Biochem Biophys Res Comm 169:1198–1203, 1990.
24. Minks MA, West DK, Benvin S, Greene JJ, Ts'o POP, Baglioni C: J Biol Chem 255:6403–6407, 1980.
25. Schröder HC, Wenger R, Rottmann M, Müller WEG: Biol Chem Hoppe-Seyler 369:985–995, 1988.
26. Sen GC, Hideharu T, Lengyel P: J Biol Chem 253:5915–5921, 1978.
27. Safer B: Cell 33:7–8, 1983.
28. Kimchi A, Zilberstein A, Schmidt A, Shulman L, Revel M: J Biol Chem 254:9846–9853, 1979.
29. Farrel PJ, Balkow K, Hunt T, Jackson J, Trachsel H: Cell 11:187–200, 1977.
30. Levin DH, London IM: Proc Natl Acad Sci USA 75: 1121–1125, 1978.
31. Galabru J, Katze MG, Nadine R, Hovanessian AG: Eur J Biochem 178:581–589, 1989.
32. Hunter T, Hunt T, Jackson RJ, Robertson HD: J Biol Chem 250:409–417, 1975.
33. Kostura M, Mathews MB: Mol Cell Biol 9:1576–1586, 1989.
34. Jacobs BL, Imani F: J Interferon Res 8:821–830, 1988.
35. Kaempfer R: Biochem Biophys Res Comm 61:591–597, 1974.
36. Harary R, Gonsky R, Itamar D, Kaempfer R: Virol 174:494–503, 1990.
37. Meegan JM, Marcus PI: Science 244:1089–1091, 1989.
38. Rebagliati MR, Melton DA: Cell 48:599–605, 1987.
39. Bass BL, Weintraub H: Cell 55:1089–1098, 1988.
40. Wagner RW, Nishikura K: Mol Cell Biol 8:770–777, 1988.
41. Nemes MM, Tytell AA, Lampson GP, Field AK, Hilleman MR: Proc Soc Exp Biol Med 132:776–783, 1969.
42. Nemes MM, Tytell AA, Lampson GP, Field AK, Hilleman MR: Proc Soc Exp Biol Med 132:784–789, 1969.
43. Chebath J, Benech P, Revel M, Vigneron M: Nature 330:587–588, 1987.
44. Charachon G, Sobol RW, Bisba C, Saehzada T, Silhol

M, Charubala R, Pfleiderer W, Lebleu B, Suhadolnik RJ: Biochemistry 29:2550–2556, 1990.
45. Horisberger MA, Staeheli P, Haller O: Proc Natl Acad Sci USA 80:1910–1914, 1983.
46. Staeheli P, Haller O: Mol Cell Biol 5:2150–2153, 1985.
47. Horisberger MA, Hochkeppel HK: J Biol Chem 260: 1730–1733, 1985.
48. Staeheli P, Yu Y-X, Grob R, Haller O: Mol Cell Biol 9:3117–3121, 1989.
49. Goetschy JF, Zeller H, Content H, Horisberger MA: J Virol 63:2616–2622, 1989.
50. Cayley PL, Davies JA, McCullagh KG, Kerr IM: Eur J Biochem 143:165–174, 1984.
51. Hersh CS, Brown RE, Roberts WK, Swyryd EA, Kerr IM, Stark GR: J Biol Chem 259:1731–1737, 1984.
52. Joklic WK, Huismans H: Virol 70:411–424, 1976.
53. Imani F, Jacobs BL: Proc Natl Acad Sci USA 85:7887–7891, 1988.
54. Kitajewski, Schneider RJ, Safer B, Munemitsu SM, Samuel CE, Thimmappaya B, Shenk T: Cell 45:195–200, 1986.
55. O'Malley RP, Mariano TM, Siekiera J, Mathews MB: Cell 44:391–400, 1986.
56. Fisher AG, Feinberg MZ, Josephs SF, Harper ME, Marselle LM, Reyes G, Gonda MA, Aldovini A, Debock C, Gallo RC, Wong-Staal FW: Nature 320:367–371, 1986.
57. Okamoto T, Wong-Staal F: Cell 47:29–35, 1986.
58. Nelbrock P, Dillon PJ, Perkins A, Rosen CA: Science 248:1650–1652, 1990.
59. Parkin NT, Cohen EA, Darveau A, Rosen C, Haseltine W, Sonnenberg N: EMBO J 7:2831–2837, 1988.
60. Edery I, Petryshyn R, Sonnenberg N: Cell 56:303–312, 1989.
61. SenGupta DN, Silverman RH: Nucleic Acids Res 17: 969–978, 1989.
62. Roy S, Katze MG, Parkin NT, Edery I, Hovanessian AG, Sonnenberg N: Science 247:1216–1219, 1990.
63. Schröder HC, Ugarkovic D, Wenger R, Reuter P, Okamoto T, Müller WEG: AIDS Res Hum Retroviruses 6:659–672, 1990.
64. Gunnery S, Rice AP, Robertson HD, Mathews MB: Proc Natl Acad Sci USA (submitted).
65. Montefiori DC, Mitchell WM: Proc Natl Acad Sci USA 84:2985–2989, 1987.
66. Montefiori DC, Pellegrino MG, Robinson E, Engle K, Feild M, Mitchell WM, Gillespie DH: Biochem Biophys Res Commun 158:943–950, 1989.
67. Montefiori DC, Robinson WE, Mitchell WM: AIDS Res Hum Retroviruses 5:193–203, 1988.
68. Lawrence J, Kulkosky J, Friedman SM, Posnett DN, Ts'o POP: J Clin Invest 80:1631–1639, 1987.
69. Schröder HC, Wenger R, Kuchino Y, Müller WEG: J Biol Chem 264:5669–5673, 1989.
70. Schröder HC, Ugarkovic D, Marz H, Kuchino Y, Okamoto T, Miller WEG: VASEB J 4:3124–3130, 1990.
71. Levy HB, Law LW, Rabson AS: Proc Natl Acad Sci USA 62:357–361, 1969.
72. Fisher JC, Cooperbrand SR, Mannick JA: Surgical Forum 21:122–124, 1970.
73. Levy HB: Arch Int Med 126:78–83, 1970.
74. Forsberg K, Paulsson Y, Westermark B: J Cell Physiol 136:266–272, 1988.
75. Zullo JN, Cochran BH, Huang AS, Stiles CD: Cell 43:793–800, 1985.
76. Vilcek J, Kohase M, Henrikson-DeStefano D: J Cell Physiol 130:37–43, 1987.
77. Hubbell HR, Liu RS, Maxwell BL: Cancer Res 44:3252–3257, 1984.
78. Chapekar MS, Knode MC, Glazer RI: Mol Pharm 34: 461–466, 1988.
79. Hubbell HR: Int. J. Cancer 37:359–365, 1986.
80. Lin SL, Greene JJ, Ts'o POP, Carter WA: Nature 297:417–419, 1982.
81. Arad G, Hershkovitz M, Panet A, Loyter A: Biochim Biophys Acta 859:88–94, 1986.
82. Hubbell HR, Boyer JE, Roane P, Burch RM: Proc Natl Acad Sci USA 88:906–910, 1991.
83. Singh VN, Singh M, August JT, Horecker BL: Proc Natl Acad Sci USA 71:4129–4132, 1974.
84. Oehler JR, Lindsay LR, Nunn ME, Holden HT, Herberman RB: Int J Cancer 21:210–220, 1978.
85. Djeu JY, Heinbaugh JA, Holden HT, Herberman RB: J Immunol 122:175–181, 1979.
86. Burleson GR, Fuller LB, Ménache MG, Grahem JA: Proc Soc Exp Biol Med 184:468–476, 1987.
87. Braun W, Nakano M: Science 157:819–821, 1967.
88. Lepe-Zuniga L, Rotbein J, Gutterman JU: J Interferon Res 9:445–456, 1989.
89. Wong GHW, Goeddel DV: Nature 323:819–822, 1986.
90. Torres BA, Johnson HM: Biochem Biophys Res Comm 131:395–402, 1985.
91. Urban JL, Shepard HM, Rothstein JL, Sugarman BJ, Schreiber H: Proc Natl Acad Sci USA 83:5233–5237, 1986.
92. Remals L, Fransen L, Huygen K, DeBaetselier P: J Immunol 144:4477–4486, 1990.
93. Fleit HB, Rabinovitch M: Cellular Immunol 57:495–504, 1981.
94. Lew RW, Herriot MJ: Cellular Immunol 106:114–121, 1987.
95. Fleit HB, Rabinovitch M: Cellular Immunol 57:495–504, 1981.
96. Riches DWH, Henson PM, Remigio LK, Catterall JF, Strunk RC: J Immunol 141:180–188, 1988.
97. Nilsen TW, Maroney PA, Robertson H, Baglioni C: Mol Cell Biol 2:154–160, 1982.
98. Suhadolnik R, Reichenbach NL, Lee C, Strayer D, Brodsky I, Carter WA: In Williams BRG, Silverman RH (eds): "The 2-5A System: Molecular and Clinical Aspects of the Interferon Regulated 2-5A System." New York: Alan R. Liss, Inc, pp 449–456, 1985.
99. Zarling J, Schlais J, Eskra L, Ts'o POP, Carter WA: J Immunol 124:1852–1857, 1980.
100. Vouge TS, Reynolds WC, Reynolds D, Fossum S and Rolstad B: Eur J Immunol 19:1895–1902, 1989.
101. Timonen T, Ortaldo JR, Heuberman RB: J Immunol 128:2514–2521, 1982.
102. Cohen SA, Salazar D, VonMuenchhausen W, Werner-Wasik M, Nolan JP: J Leukocyte Biol 37:559, 1985.
103. Santoni A, Gismonde A, Morrone S, Procopio A, Modesti A, Scarpa S, D'Orazi G, Piccoli M, Frati L: J Immunol 143:2415–2421, 1989.
104. Tamura-Nishimura M, Sasakawa S: FEBS Letters 248: 73–77, 1989.
105. Fan XD, Goldberg M, Bloom BR: Proc Natl Acad Sci USA 85:5122–5125, 1988.
106. Braun W, Nakano M: Proc Soc Exp Biol Med 119:701–707, 1965.

107. Turner W, Chan SP, Chirigos MA: Proc Soc Exp Biol 133:334–338, 1970.
108. Cunnington PG, Naysmith JD: Immunol 29:1001–1017, 1975.
109. Abruzzo LV, Mullen CA, Rowley DA: Cellular Immunol 98:266–278, 1986.
110. Shuttleworth J, Morser J, Burke DC: Eur J Biochem 133:399–404, 1983.
111. Krown SE, Friden GB, Khansur T, Davies ME, Oettegen HF, Field AK: J Interferon Res 3:281–289, 1983.
112. Strauss KI, Strayer DR, Gillespie DH: J Pharm Pharmacol 42:261–266, 1989.
113. Carter WA, Pitha PM, Marshall LW, Tazawa I, Tazawa S, Ts'o POP: J Mol Biol 70:567–587, 1972.
114. Strayer DR, Watson P, Carter WA, Brodsky I: J Interferon Res 6:373–379, 1986.
115. Carter WA, Hubbell HR, Krueger LJ, Strayer DR: J Biol Resp Mod 4:613–620, 1985.
116. Strayer D, Brodsky I, Peguignot E, Crilley P, Carter W, Fenning R, Kariko K, Reichenbach N, Sobol RW, Li SW, Suhadolnik R: In Diasio RB, Sommadossi JP (eds): "Advances in Chemotherapy of AIDS." New York: Pergamon Press, pp 23–31, 1990.
117. Strayer DR, Carter WA, Pequignot E: manuscript in preparation.
118. Wu JM, Chaio JW, Maayan A: AIDS Res 2:127–131, 1986.
119. Carter WA, Strayer DR, Brodsky I, Lewin M, Pelligrino MG, Einck L, Henriques HF, Simon GL, Parenti DM, Scheib RG, Schulof RS, Montefiori DC, Robinson WE, Mitchell WM, Volsky DJ, Paul D, Paxton H, Meyer WA III, Karikó K, Reichenbach N, Suhadolnik RJ, Gillespie DH: Lancet i:1286–1292, 1987.
120. Preble OT, Rook AH, Steis R, Silverman RH, Krause D, Quinnan GU, Masur H, Jacob J, Lango D, Gelmann EP: J Inf Dis 152:457–465, 1985.
121. Suhadolnik RJ, Kariko K, Reichenbach NL: manuscript in preparation.
122. Hall DJ, Jones SD, Kaplan DR, Whitman M, Rollins BJ, Stiles CD: Mol Cell Biol 10:2448–2457, 1990.
123. Wathelet MG, Clauss IM, Nols CB, Content J, Huez GA: Eur J Biochem 169:313–321, 1987.
124. Wathelet MG, Clauss IM, Content J, Huez GA: Eur J Biochem 174:323–329, 1988.
125. Ray A, Tatter SB, May LT, Sehgal PB: Proc Natl Acad Sci USA 85:6701–6705, 1988.
126. Hiscott J, Cantell K, Weissmann C: Nucl Acids Res 12:3727–3745, 1984.
127. Varnum BC, Lim RW, Herschman HR: Oncogene 4:1205–1265, 1989.
128. Muesing MA, Smith DH, Capon DJ: Cell 48:691–701, 1987.
129. Ringold GM, Dieckmann B, Vannice JL, Trahey M, McCormick F: Proc Natl Acad Sci USA 81:3964–3968, 1984.
130. Paez E, Esteban M: Virol 134:12–28, 1984.
131. Duh EJ, Maury WJ, Folks TM, Rabson AB: Proc Natl Acad Sci USA 86:5974–5978, 1989.
132. Montefiori DC, Robinson WE, Mitchell WM: AIDS Res Hum Retriviruses 5:193–203, 1988.
133. Kleinerman E, Licht R, Gallo R, Fidler J: Cancer Res 44:4470–4475, 1984.
134. Tamura-Nishimura M, Saito S, Sasakawa S: J Interferon Res 5:77–84, 1985.
135. Tamura-Nishimura M, Sasakawa S: FEBS Letters 261: 343–346, 1990.