INTERFERON: EFFECTS ON THE IMMUNE RESPONSE AND THE MECHANISM OF ACTIVATION OF THE CELLULAR RESPONSE

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INTRODUCTION

The discovery of interferon in 1957 by Drs. Alick Isaacs and Jean Lindenmann led to major revisions in the concepts of man's defenses against viral infections. Interferons are well known as inhibitors of virus replication. This inhibition is accomplished by acting on the host cell rather than on the virus (Figure 1). The proposed biological mediator of the antiviral action of interferon is not interferon, but an antiviral protein, which is produced by a cell after that cell has come into contact with interferon.

Several substances, including viruses, are capable of stimulating the production of interferon* in mammalian cells. Viruses and nucleic acids are capable of stimulating the production of interferons in virtually all nucleated cell types, while antigens and mitogens that are reactive with thymus-derived or T lymphocytes primarily stimulate interferon production in lymphocytes.[1] Since interferons are produced by antigen- and mitogen-activated lymphocytes, it is possible that they may have natural biological functions related to immunity. There is a growing body of evidence suggesting that viral and antigen-induced interferons have a modulating effect on the immune response. Recently, the T lymphocyte, along with its "helper" function, has been shown to have a suppressive effect on the immune response.[2] Evidence will be presented here that suggests a possible natural role for interferons in the mediation of so-called suppressor T cell effects.

Data will also be presented on the cellular

*It is becoming clear that, depending upon the inducer and the stimulated cell, there are several types of interferons that can be produced by mammalian cells. The following is defined for operational purposes: (1) Virus-induced (virus-type) interferon. This term is used for interferon produced in lymphoid or nonlymphoid cells under the stimulation of virus or double-stranded RNA. This interferon is sometimes called Type I interferon in the murine system. (2) Mitogen-induced (mitogen-type) interferon. This term is used for interferon produced in lymphoid cells under the stimulation of T lymphocyte mitogens. This interferon is antigenically distinct from virus-induced (virus-type) interferon. (3) Antigen-induced (antigen-type) interferon. This term is used for interferon produced in antigen-primed lymphoid cells upon second exposure to the specific antigen. This interferon is also antigenically distinct from virus-induced (virus-type) interferon, and it is sometimes called Type II interferon in the murine system. It is tentatively felt that mitogen- and antigen-type interferons are the same in the murine system, since no evidence to the contrary is presently available.

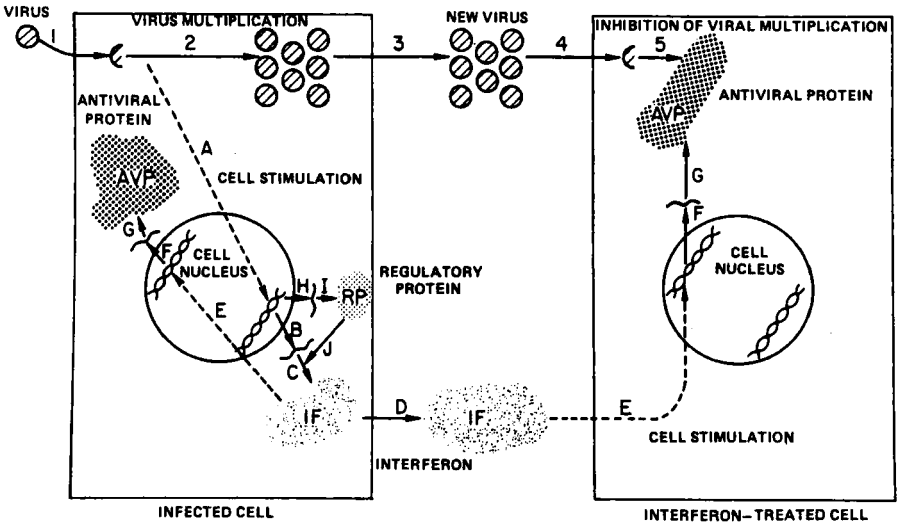


FIGURE 1. Cellular events of the induction and action of interferon (IF). Virus comes in contact with the cell (1) and penetrates the cell membrane. The virus then releases its genetic material, and replication of the virus occurs (2). The new virus leaves the cell (3), enters the fluid around the first cell, and some of the replicated virus infects a second cell (4), where the release of the genetic material again takes place (5). During the early stages of infection of the first cell, some event (viral nucleic acid?) stimulates a gene in the DNA which contains the stored genetic information for interferon (A). This leads to the production of a messenger RNA for interferon, which leaves (B) the nucleus and is translated by the cell's ribosomes (C) into the interferon protein. Several events now occur more or less simultaneously. Some interferon is secreted by the first cell (D), enters the surrounding fluid, where it comes into contact with and stimulates the second cell (E). The second cell is thereby induced to produce a new messenger RNA (F) which is translated to a new protein(s) (G), the antiviral protein (AVP). This in turn modifies the cell's protein-synthesizing machinery, such that cell mRNA is translated into protein, but viral RNA is poorly bound or translated, or both. In the first cell processes E, F, and G may, in some instances, also operated to form AVP and thereby reduce the virus yield in the first cell. Shortly after interferon is synthesized into the first cell, another mRNA (h) is believed to be synthesized from the cell's DNA which is translated (1) into a regulatory protein (RP), (hypothesized). This regulatory protein combines with the mRNA for interferon, thereby preventing the further synthesis of more interferon (J). (From DHEW Publ. No. (NIH) 75-700).

events involved in the activation of a cell by virus-induced (virus-type) interferon, resulting in the production of the antiviral protein. Specifically, the following will be examined: (1) the cellular site of action of interferon; (2) the nature of the cellular receptor for interferon; (3) the nature of the mechanism of interferon derepression of the gene for the production of the antiviral protein; (4) the chromosomal location of the genes controlling the interferon system; and (5) the cell regulatory machinery for the production of the antiviral protein. We will not cover the mechanism of the discriminatory effect of the antiviral protein in specifically blocking virus replication. This aspect of the antiviral effects of interferon has been recently reviewed elsewhere.[2,3]

The approach in this rapidly emerging aspect of interferon research is interdisciplinary. Interferons are finally being examined for their broad and general regulatory roles in cell functions, and the implications of the findings are such that virologists, immunologists, and others interested in cell function will have a broader appreciation for the role of interferon in regulating cell functions.

THE REGULATORY ROLE OF INTERFERONS IN THE IMMUNE RESPONSE

The In Vivo Antibody Response

Some investigators have observed a moderate enhancement of the plaque-forming cell (PFC) response to sheep red blood cells (SRBC) in mice treated within certain dose ranges of virus-type interferon, while higher dosages resulted in modest suppression of the response.[5] It was suggested that

the modulating effects of interferon preparations on the immune response may have been due to modifications of the cyclic adenosine monophosphate (cAMP) system. Preliminary data demonstrated that mouse interferon preparations were capable of elevating adenyl cyclase activity and cAMP levels in mouse spleen cells. Detailed studies correlating the biological effects of the interferon preparations with their cAMP stimulating effects were not presented, hence the above-mentioned relationships would appear to be conjecture. Studies with dibutyryl cAMP and mouse L cells have shown that large amounts of the cyclic nucleotide were capable of blocking the production of interferon induced either by polyriboinosinate·polyribocytidylate or by Newcastle disease virus.[6] The precise relationships, then, between the various biological properties of interferons, including their effects on the immune response, and the cyclic ribonucleotide system will require a great deal more study.

Other investigators[7,8] have shown that if mice were injected with 1.5×10^5 units or more (Figure 2) of virus-type interferon over a 4-hr period, the antibody response to SRBC was significantly suppressed. The inhibition was most effective if interferon was administered 2 days prior to antigen injection. It is of interest that the dosage of interferon required for suppression was 20 times that of the studies reported above.[5] Dosages of human interferon comparable to those of mouse inhibitory dosages had no effect on the mouse splenic PFC response, indicating that the phylogenetic specificity of the antiviral effect of interferon also extended to its in vivo PFC inhibitory effect. Inactivation of interferon by heating or trypsin resulted in a corresponding decrease in its PFC inhibitory effect.

The ability of virus-type interferon to inhibit the antibody response in mice was found to be related to the antigen dose.[8] The antibody response of mice to 1×10^7 SRBC, for example, was significantly inhibited by 1.5×10^5 units of interferon, while the response to 2×10^8 SRBC was unaffected by the same amount of interferon. Both the primary and secondary antibody responses as well as IgM and IgG antibody synthesis were affected.

The SRBC is a thymus-dependent antigen requiring the cooperation of both B and T lymphocytes for optimal antibody production.[9] It was found that the in vivo antibody response to *Salmonella typhimurium* lipopolysaccharide (LPS), a thymus-independent antigen, was also significantly inhibited by virus-type interferon preparations.[10] The immunosuppressive activity of interferon was both time- and dose-dependent. Maximum suppression was produced when mice were given 1.5×10^5 units of interferon 2 days prior to LPS stimulation. The authors suggested that interferon affects some early event(s) in the process of antibody synthesis that might be related to the general inhibitory effect of interferon on rapidly dividing cells and messenger RNA (mRNA) translation. By removing the adherent cells from spleen cells of mice treated with interferon and stimulation of the nonadherent cells by LPS in vitro, data were obtained that suggested that interferon can act directly on B lymphocytes.

The In Vitro Antibody Response

The in vivo studies require large amounts of interferon, and the concentration and kinetics of administered interferon in the microenvironment of the immunocompetent cell are unknown. For this reason, data on the effect of interferon on the in vitro PFC response are of interest. In one series of studies, approximately 3,000 units of virus-type L cell interferon per milliliter were required to significantly suppress the in vitro PFC response of BALB/c mouse spleen cells to SRBC (Figure 3).[11] The effect was seen when the cultures were pretreated with interferon for 6 hr, or when interferon was added up to 40 hr after addition of SRBC. The factor responsible for the inhibition could not be dissociated from the antiviral activity of interferon either by specific activity of the interferon or by standard physicochemical means, such as treatment of interferon with trypsin, periodate, RNAase, and DNAase. Trypsin and periodate treatment, which destroyed the interferon, also destroyed the PFC inhibitory effect of the interferon preparations. By the use of separated cell populations, it was shown that interferon acted directly on B lymphocytes and had no effect on macrophages or T lymphocyte helper effect. This latter observation regarding T cell helper effect is significant, since it is the only T cell function reported not to be affected by interferon, and therefore needs to be investigated further. The above in vitro study required lesser amounts of interferon for PFC inhibition than did the in vivo studies, but the inhibitory concentrations were considerably higher than the physiological concentrations normally obtained by stimulation of cultures with interferon inducers.[1]

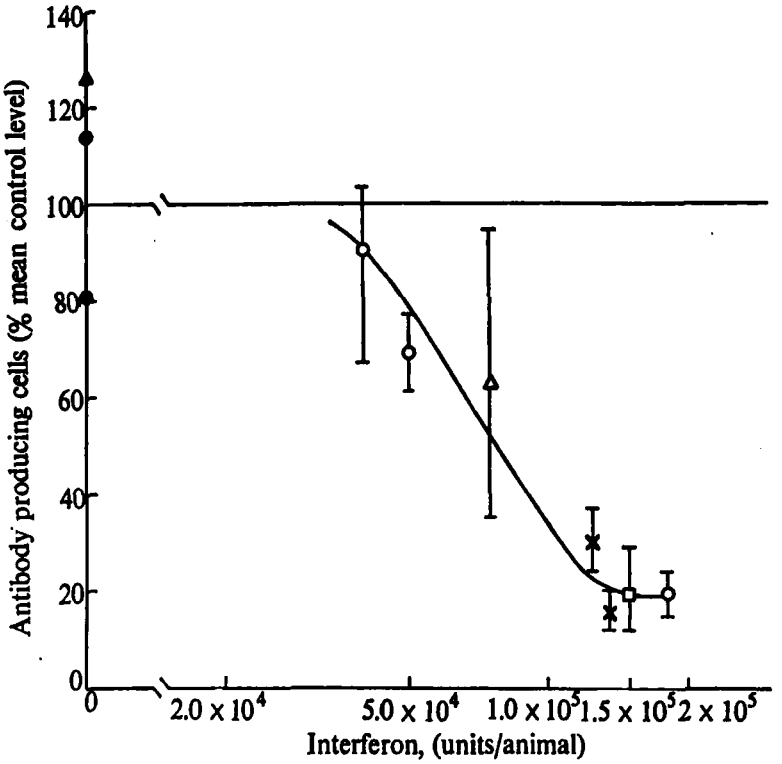


FIGURE 2. Relationship of interferon dose and number of antibody-producing spleen cells. Animals were sensitized to SRBC antigen by intravenous administration of 2 × 10^7 SRBC in 0.5 ml saline. Antibody-producing capability was measured by the number of spleen cells producing antibody to SRBC 6 days after antigen administration. Various doses of interferon were administered 2 days before administration of antigen (SRBC). The number of nucleated spleen cells producing antibody to SRBC was assayed 6 days after antigen administration and expressed as a percentage of the number of antibody-producing spleen cells in uninjected controls. All determinations shown represent the mean in at least two animals. Ranges shown are ± SD. Two uninduced control preparations containing less than 10 units of interferon were also used. One consisted of pressure dialyzed supernatant of L-929 cell tissue culture medium (•), and the other of serum from untreated mice (▲). Interferon preparations from four sources were used, all induced with NDV. One from L-929 cells and pressure dialyzed 5 to 50-fold (○), one from C243-3C cells and pressure dialyzed 20-fold (x), one from mouse serum 10 hr after injection of NDV (△), and one purified on an anti-interferon affinity chromatography column (□). Five days of pH 2 treatment was used to inactivate NDV in all interferon preparations, and no residual interfering activity as titered in human neonatal fibroblasts was noted in any preparation. (From Chester, T. J., Paucker, K., and Merigan, T. C., *Nature*, 246, 92, 1973. With permission.)

Others[12,13] have demonstrated with C57B1/6 mouse spleen cells that 20 to 60 units of virus-type interferon from various sources inhibited the in vitro PFC response to SRBC by 90% or more. An example of one such inhibition is presented in Figure 4. Greater than 99% inhibition of the PFC response was obtained with 40 or more units of crude L cell interferon/culture. PFC inhibition data with partially purified mouse L cell and mouse ascites tumor interferons are presented in Figure 5. L cell interferon (50 units/culture) inhibited the anti-SRBC PFC response by 87% (Figure 5A), while ascites tumor interferon (20 units/culture) inhibited the PFC response by 92% (Figure 5B). These inhibitory concentrations of partially purified interferons did not affect viable cell recovery. Although the above interferon preparations were of different potencies and specific activities, they inhibited the PFC response in proportion to their antiviral activities. In addition,

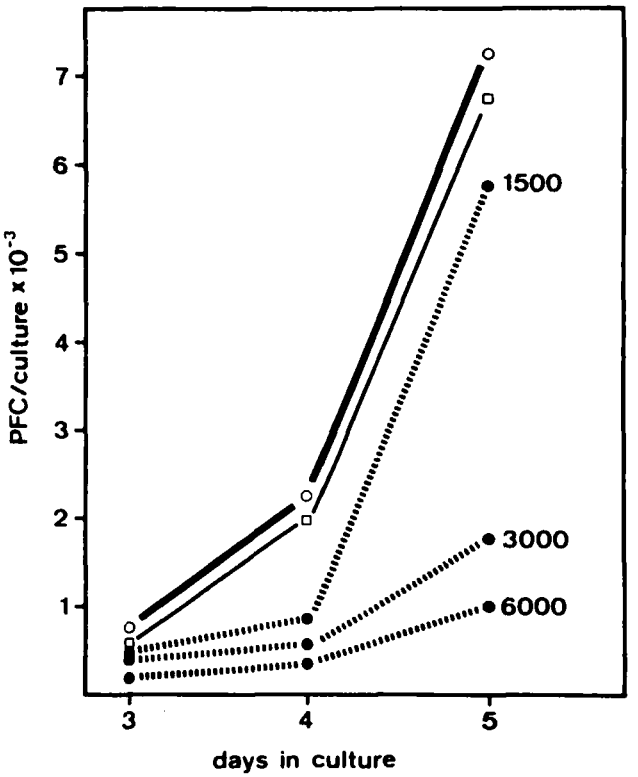


FIGURE 3. Effect of interferon on anti-SRBC plaque formation in spleen cell cultures. Indicated amounts of mouse L cell interferon (units/ml) were added together with antigen. ○—○, untreated cultures; □—□, cultures treated with control preparation; ●---●, interferon-treated cultures. (From Gisler, R. H., Lindahl, P., and Gresser, I., *J. Immunol.*, 113, 438, 1974. With permission. © 1974, The Williams & Wilkins Co., Baltimore.)

both the antiviral activity and the PFC inhibitory activity of the interferons were neutralized by antibody specific for mouse interferon. Both activities were partially or completely inactivated by heating at 60°C for 1 hr. Human interferons had neither antiviral activity nor PFC inhibitory activity in mouse cells. Limited exposure (4 hr) of cells to interferons significantly inhibits both viral infection and the PFC response. Both the antiviral activity and the PFC inhibitory activity of the interferon preparations are acid stable. It was concluded, therefore, that the inhibition of the primary in vitro PFC response was due to interferon in the preparations.[13]

Kinetic data (Figure 6) showed that the greater the concentration of interferon added to the cultures, the earlier the effect on the PFC response.[13] Also, the presence of interferon in the culture for the first 4 hr was sufficient to inhibit the PFC response. Interferon, then, appeared to affect some early event(s) which lead to inhibition of the PFC response. These events do not appear necessarily to involve antigen "processing" by macrophages or induction of lymphocytes by "processed" antigen, since the kinetic data showed that the B cells could be induced by SRBC to produce PFC on days 3 and 4 in studies involving low dosages of interferons (50 units/culture).

The effect of virus-type interferon on the in vitro PFC response to a thymus- and macrophage-independent antigen has been studied.[14] It required about twice as much interferon (100 to 200 units) to inhibit the PFC response to *E. coli* 0127 LPS as it did to inhibit the anti-SRBC response (Figure 7A). By the use of spleen cells from nude (athymic) mice (Figure 7B) and spleen cells depleted of macrophages (Figure 8), it was shown that T lymphocytes and macrophages were not required for interferon to exert its inhibitory effect.

Virus-type interferon inhibition of the primary in vitro PFC response in C57Bl/6 mice involves a

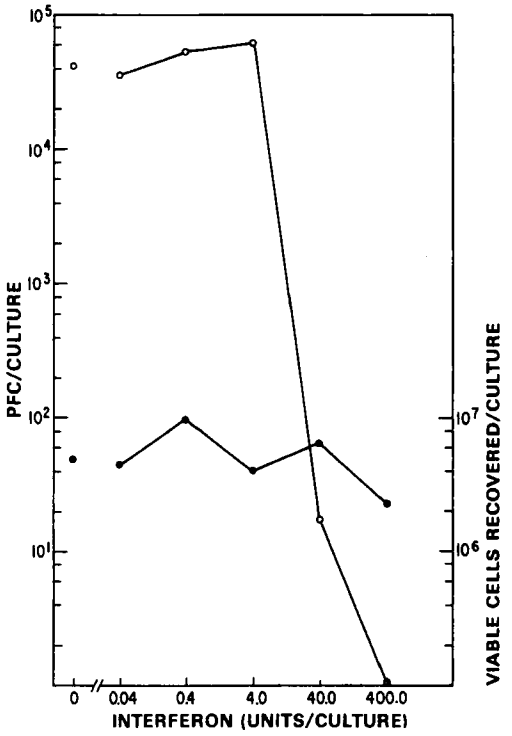


FIGURE 4. The effect of crude mouse L cell interferon on the primary in vitro PFC response to SRBC. Direct anti-SRBC PFC per culture (o—o) and viable cells recovered per culture (•—•) were determined on day 5. (From Johnson, H. M., Smith, B. G., and Baron, S., *J. Immunol.*, 114, 403, 1975. With permission. © 1975, The Williams & Wilkins Co., Baltimore.)

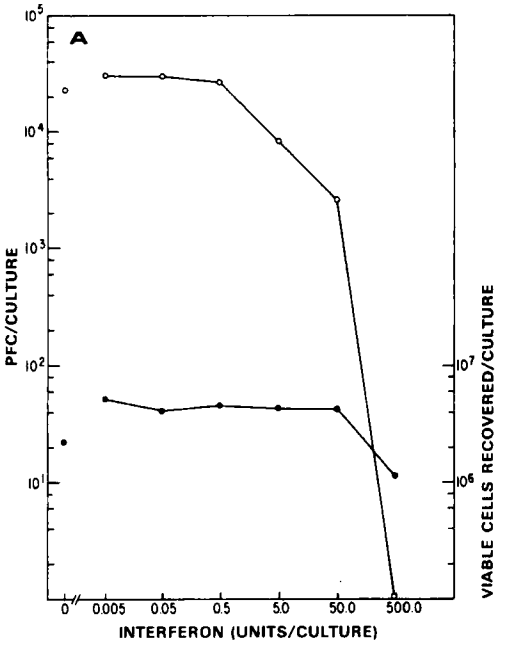
dynamic relationship between the nature of the antigen, the concentration of interferon added to antigen-stimulated cultures, and the time of addition of interferon relative to antigen addition.[15] The PFC response to a thymus-dependent antigen, SRBC, was more easily suppressed by interferon than was that to a thymus-independent antigen, *E. coli* 0127 LPS, both in terms of inhibitory concentrations of interferon and the time at which the interferon could be added to cultures after antigen and still inhibit the PFC response (Figure 9). The anti-SRBC response was effectively inhibited by 150 units of interferon when the interferon was added to mouse spleen cultures up to 16 hr (87% inhibition) after SRBC. At 500 units of interferon, effective inhibition (76%) was obtained when the interferon was added up to 24 hr after SRBC. In the anti-*E. coli* 0127 response, 150 units of interferon effectively inhibited the PFC response only when added at the time of antigen, and not 8 hr later. With 500 units of interferon, effective inhibition (70%) was obtained when interferon was added to cultures up to 16 hr after *E. coli* 0127. The anti-SRBC PFC response was inhibited more extensively by both 150 and 500 units of interferon than was the anti-*E. coli* 0127 response when interferon was added to culture at either 8, 16, or 24 hr after antigen.

Evidence has been presented that suggests that splenic memory lymphocytes represent a cell population that differs qualitatively from that of unstimulated spleen cells.[16] Virus-type interferon has been shown[15] to be effective in inhibiting (91% with 120 units of interferon) the generation of PFC from this memory cell pool (Figure 10). Both virgin and memory lymphocyte populations, then, are inhibited by virus-type interferon in the in vitro PFC response. This is in agreement with the inhibitory effects of interferon on the secondary antibody response in in vivo studies.[8] It is of interest that the in vitro PFC inhibition of memory lymphocytes was obtained with physiological concentrations of interferon.

Both the in vivo and in vitro studies cited above present data that suggest that under certain conditions interferon may also exert a small enhancing effect on the antibody response in addition to its suppressive effects. Injections of interferon into mice or additions to mouse spleen culture 2 to 3 days after antigen have been shown to slightly enhance the antibody response.[10,13] Similarly, suboptimal immune responses in vitro have been elevated to optimal levels by interferon.[11] Some enhancement has also been noted with low concentrations of interferon.[5] The enhancement data are not as impressive as the data showing the immunosuppressive effects of interferon. Some of the in vitro studies[13] reported above have recently been confirmed.[15]

Cellular Immunity and Delayed-type Hypersensitivity

Along with its inhibitory effects on antibody production, virus-type interferon has also been shown to inhibit cellular immune responses or delayed-type hypersensitivities. Interferon (2,500 units/ml) was shown to inhibit DNA synthesis of phytohemagglutinin-stimulated and mixed lymphocyte-stimulated mouse spleen cell suspensions.[18] It is well known that these effects involve the T or thymus-derived lymphocytes. A similar inhibitory effect has been noted on concanavalin A-stimulated lymphocytes.[19] Interferon



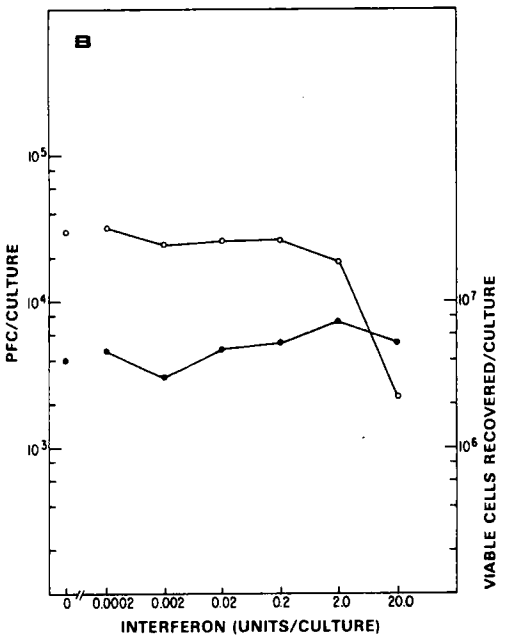


FIGURE 5. The effect of partially purified mouse L cell (A) (1 X 10^7 NIH reference units/mg protein) and mouse ascites tumor (B) (3.2 X 10^8 NIH reference units/mg protein) interferons on the primary in vitro PRC response to SRBC. Direct anti-SRBC PFC per culture (○—○) and viable cells recovered per culture (●—●) were determined on day 5. (From Johnson, H. M., Smith, B. G., and Baron, S., *J. Immunol.*, 114, 403, 1975. With permission. © 1975, The Williams & Wilkins Co., Baltimore.)

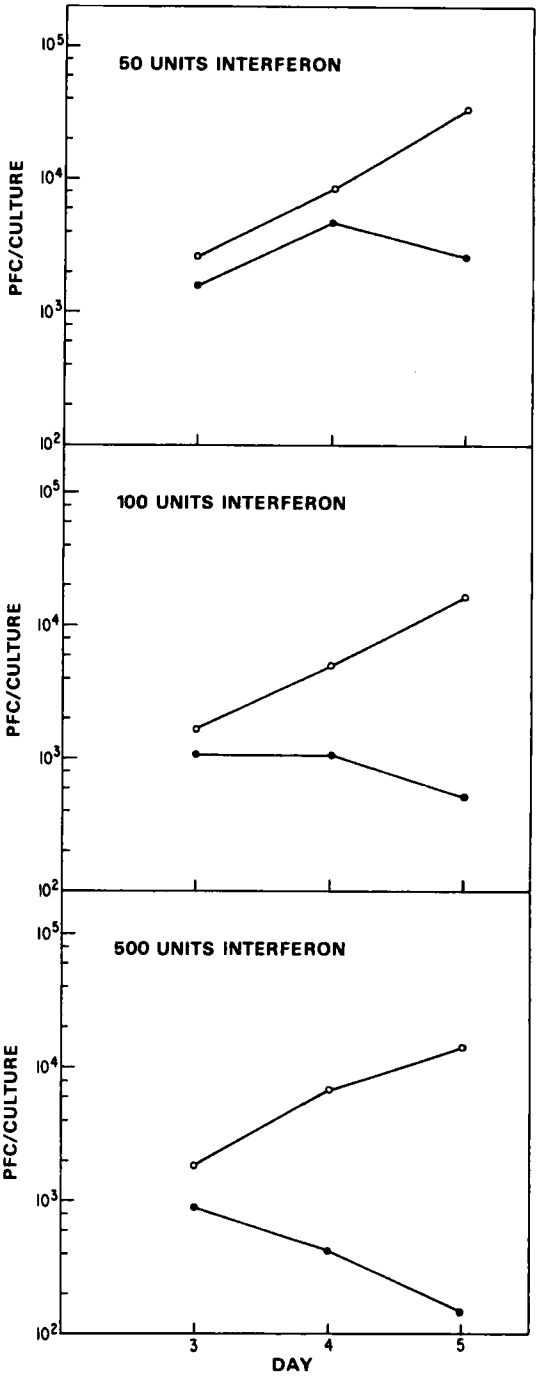


FIGURE 6. Kinetics of direct anti-SRBC PFC response of mouse spleen cell cultures in the presence (●—●) or absence (○—○) of 50, 100, and 500 NIH reference units of crude L cell interferon. Interferon and SRBC were added to cultures at the same time. (From Johnson, H. M., Smith, B. G., and Baron, S., *J. Immunol.*, 114, 403, 1975. With permission. © 1975, The Williams & Wilkins Co., Baltimore.)

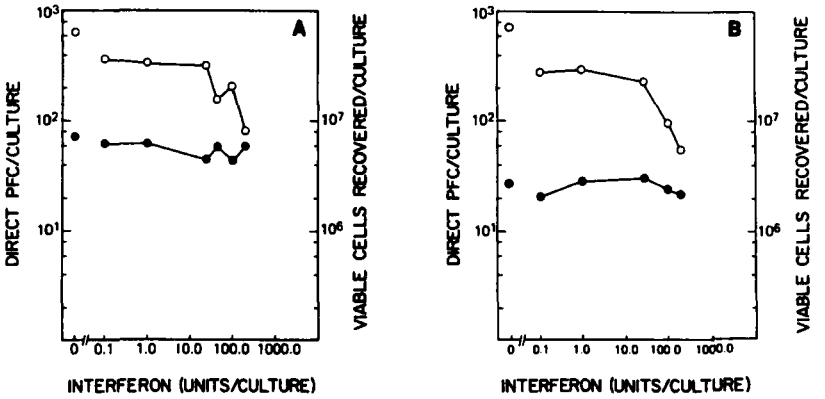


FIGURE 7. The effect of mouse crude L cell interferon on the primary in vitro PFC response to *E. coli* 0127 LPS in C57B1/6 (A) and nude (athymic) (B) mouse spleen cultures. The interferon was added at the same time as antigen, and direct anti-*E. coli* 0127 LPS PFC per culture (o—o) and viable cells recovered per culture (•—•) were determined on day 5. (From Johnson, H. M., Bukovic, J. A., and Baron, S., *Cell. Immunol.*, 20, 104, 1975. With permission.)

also inhibited the cellular immune response of mice to allografts.[20-22] Mice with contact sensitivity to picryl chloride and delayed-type hypersensitivity to SRBC were suppressed by 3.6 × 10^5 and 2.1 × 10^6 units of interferon per milliliter, respectively, when the interferon was given just prior to challenge with the specific antigens.[23] Sensitization with SRBC was also blocked by virus-type interferon and inducers of virus-type interferon.[24] T cell function, both afferent and efferent, then, is also affected by interferon.

Interferon Inducers

In contrast to the general suppressive activity of interferons, several types of interferon inducers have been reported to have both enhancing and suppressive effects on the antibody response in vivo and in vitro. Synthetic double-stranded polyribonucleotides generally enhance the immune response in mice when administered to animals along with antigens, but if given 12 to 48 hr before antigen they exert a profound suppressive effect on antibody production (see References 13 and 25). This effect is illustrated in Table 1, where mice were injected intravenously with polyriboadenylate·polyribouridylate (poly rA·poly rU) at various times in relation to intravenous injection of bovine gamma globulin (BGG).[26] Poly rA·poly rU, given 1 day to 12 hr before antigen, profoundly suppressed the antibody response. When given 2 hr before, or at the same time as antigen, a significant enhancement of the antibody response was observed.

TABLE 1

Effect of Time of Injection of Poly rA·Poly rU in Relation to Antigen

Products injected: BGG (1.0 mg) + poly rA·poly rU (300 μg)	Antibody titer (reciprocal) on day 10
−3 days[a]	640
−2 days	320
−1 day	10
−18 hr	0
−12 hr	0
−6 hr	320
−2 hr	5,120
with	2,560
+12 hr	640
+24 hr	320
BGG (1.0 mg) alone	640

[a]Time of antigen injection = day zero.

From Schmidtke, J. R. and Johnson, A. G., *J. Immunol.*, 106, 1191, 1971. With permission. © 1971, The Williams & Wilkins Co., Baltimore.

In vitro, poly rA·poly rU and polyriboinosinate·polyribocytidylate (poly rI·poly rC), at 0.1 to 1.0 μg/ml, inhibited the anti-SRBC PFC response (Figure 11) in spleen cell cultures by greater than 90% when the polyribonucleotides were added to cultures along with antigen.[25] Functional T lymphocytes were required in the cultures for the polyribonucleotides to be effective as inhibitors, thus demonstrating the thymus dependence of the inhibitions. This is illustrated in Figure 12, where poly rA·poly rU was used to

TABLE 2

Neutralization by Anti-Mouse Interferon Antibody of Inhibitory Effect of Poly rA·Poly rU on In Vitro PFC Response of C57B1/6 Spleen Cells to SRBC

Antiserum, dilution[a]	Poly rA·poly rU[a] (2 μg/culture)	Anti-SRBC PFC/culture (mean ± SD for duplicates)	% Inhibition relative to control
None	–	26,750 ± 1,061	
None	+	1,075 ± 742	96
Anti-interferon, 1:100	–	12,925 ± 177	
Anti-interferon, 1:100	+	16,650 ± 530	–29
Anti-interferon, 1:1000	–	21,550 ± 636	
Anti-interferon, 1:1000	+	18,325 ± 2,298	15
NRS,[b] 1:100	–	12,925 ± 1,237	
NRS, 1:100	+	2,400 ± 283	81

[a]Antisera and poly rA·poly rU were added to cultures at the same time. Antisera dilutions represent final concentrations after addition to culture.
[b]Normal rabbit serum (NRS) is a pool of four normal rabbit sera.

From Johnson, H. M. and Baron, S., *Cell. Immunol.*, 25, 106, 1976. With permission.

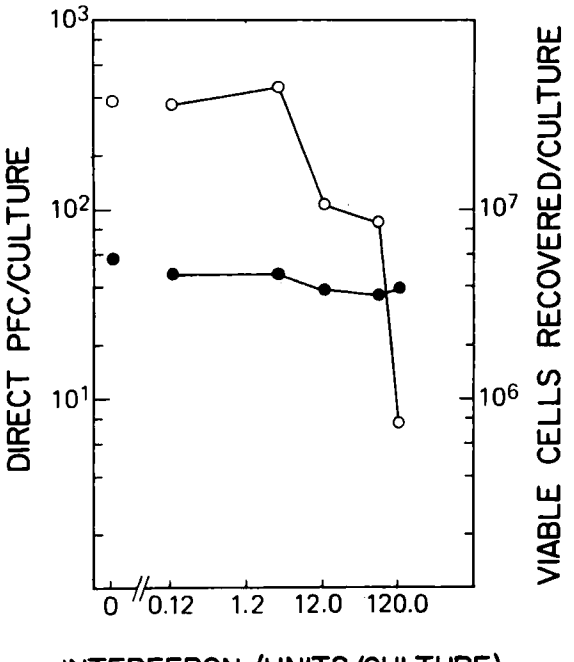


FIGURE 8. The effect of partially purified mouse L cell interferon (1 × 10^7 NIH reference units/mg protein) on the primary in vitro PRC response to *E. coli* 0127 LPS in macrophage-depleted C57B1/6 mouse spleen cultures. The interferon was added at the same time as antigen, and direct anti-*E. coli* 0127 LPS PFC per culture (o—o) and viable cells recovered per culture (•—•) were determined on day 5. (From Johnson, H. M., Bukovic, J. A., and Baron, S., *Cell. Immunol.*, 20, 104, 1975. With permission.)

inhibit the PFC response to the T-independent antigen *E. coli* 0127 LPS in C57B1/6 mouse spleen cultures and in nude (athymic) mouse spleen cultures. At 10 μg per culture, poly rA·poly rU inhibited the response in C57B1/6 cultures by 84%, while having no effect in the nude spleen cultures. The C57B1/6 cultures contained both functional B and T cells, while the nude cultures contained functional B cells, but lacked functional T cells. Ten to 100 times more polyribonucleotide was required in order to inhibit the in vitro PFC response to the T-independent antigen *E. coli* 0127 LPS than was required for the T-dependent SRBC antigen. This latter observation is further evidence of a differential effect of interferon and interferon inducers on the in vitro PFC response of mouse spleen cells to a T-dependent and T-independent antigen. Data in Table 2 show that the inhibitory effect of poly rA·poly rU on the in vitro PFC response to SRBC was neutralized by antibody to virus-type interferon.[15] The same effect was observed with *E. coli* 0127 LPS. The antiviral property of interferon stimulated in spleen cultures by polyribonucleotide is also neutralized by the same antibody to interferon. The above findings suggest that the in vitro immunosuppressive effect of double-stranded polyribonucleotides is due to their early stimulation of virus-type interferon production by T lymphocytes.

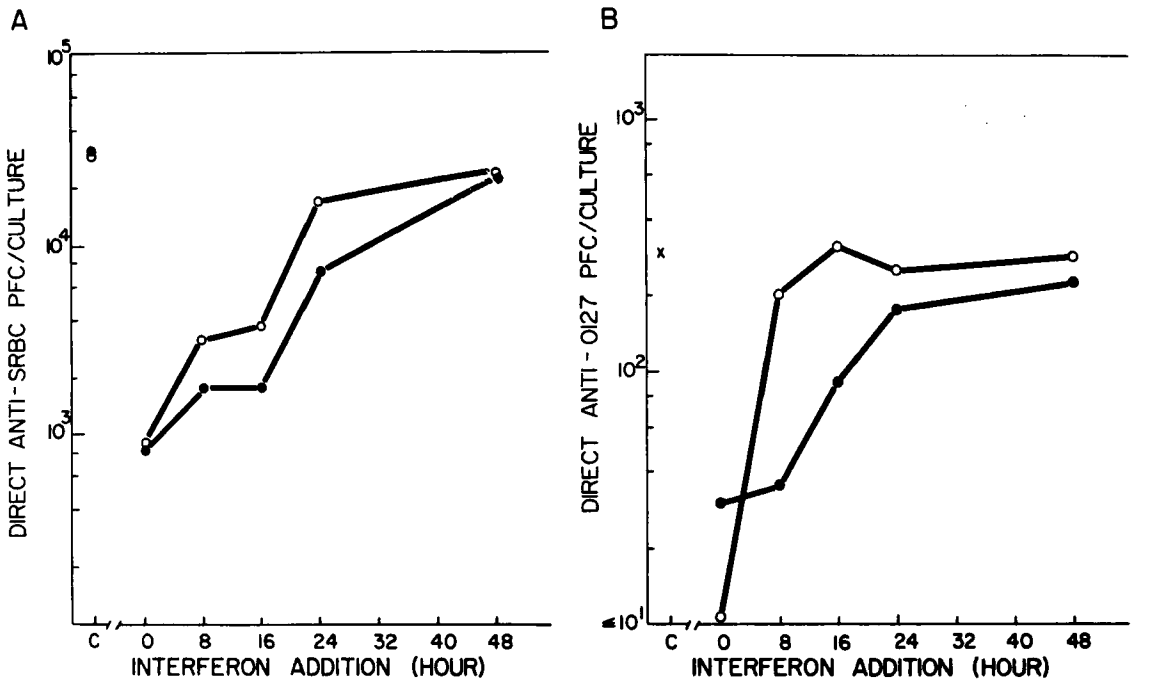


FIGURE 9. Effect of addition of 150 (o—o) and 500 (•—•) NIH reference units of viral-induced mouse L cell interferon to mouse spleen cultures at different times on the direct anti-SRBC (A) and anti-*E. coli* 0127 (B) PFC response. (From Johnson, H. M. and Baron, S., *Cell. Immunol.*, 25, 106, 1976. With permission.)

Several T lymphocyte mitogens (concanavalin A, phytohemagglutinin, and staphylococcal enterotoxin A) have been shown to stimulate interferon production only in lymphoid cell cultures[28] (see Reference 27 for review) and to suppress the antibody response of mice both in vivo[29-31] and in vitro.[32-35] Enhancement of the PFC response has been noted in rabbits when the mitogen and antigen were given simultaneously.[36] Staphylococcal enterotoxin A is about ten times more effective, on a weight basis, in inhibiting the anti-SRBC in vitro PFC response than concanavalin A (con A) (Figures 13 and 14).[35] Further, enterotoxin A is a superior inducer of mitogen-type interferon than either con A or phytohemagglutinin P (H. M. Johnson, unpublished data). The ability of T cell mitogens to inhibit the in vitro PFC response of mouse spleen cells, then, is related to their ability to stimulate the cultures to produce mitogen-type interferon. Data suggest that mitogen-stimulated peripheral T lymphocytes are the main source of early (12 hr to 3 days) produced interferon both in mice[37] and humans.[38] The subject of mitogen and antigen stimulation of interferon production by lymphocytes and the augmentation of this phenomenon by macrophages have recently been reviewed.[39] T cell mitogens also stimulated late (5 to 7 days) interferon production in B lymphocytes from humans.[38] In this regard, studies cited earlier in this review suggest that it is the interferon produced or introduced early in the in vitro antibody cultures that exerts the inhibitory effects on the antibody response. T cell mitogen (con A and enterotoxin A) induced mouse interferon was not neutralized by antibody to virus-type mouse interferon; nor were the in vitro anti-SRBC and anti-*E. coli* PFC inhibitory effects of these mitogens neutralized by the same antibody.[15] This is just the opposite of the above observations with the interferon inducer poly rA·poly rU.

The T cell mitogen-induced interferon, then, may be the same or related to antigen-type interferon, which is antigenically distinct from virus-type interferon.[40-42] We tentatively classify the mitogen- and antigen-type interferons as being the same. Thus mitogen (antigen)-type interferon, which is produced along with other lymphokines in the microenvironment of the stimulated lymphocyte, may also be able to suppress the immune response.

Interferon Inducers and the Graft-Versus-Host (GVH) Reaction

Poly rI·poly rC was employed in studies with mice to determine its effect on the GVH

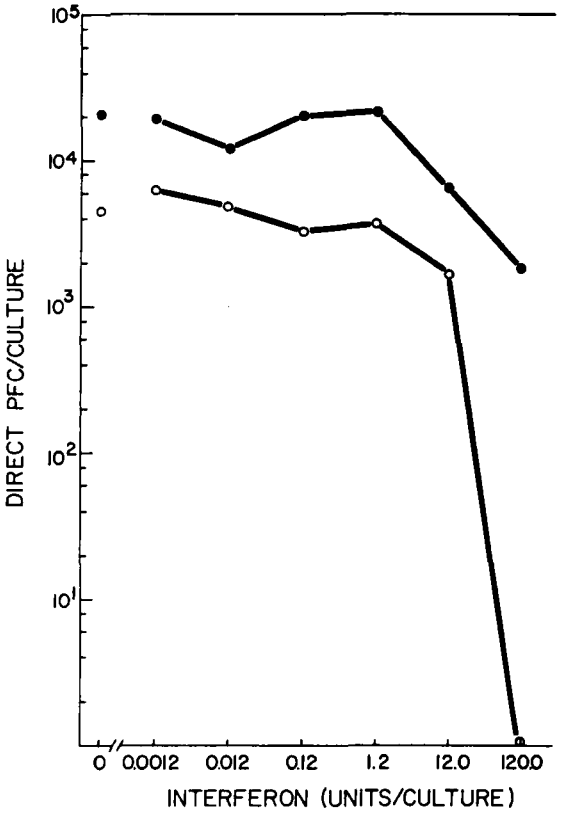


FIGURE 10. The inhibitory effect of mouse L cell interferon on the anti-SRBC PFC response of unprimed (o—o) and SRBC-primed (•—•) mouse spleen cells. Primed cells were obtained from mice injected intravenously 6 days previously with 0.1 ml of a 1% SRBC suspension. SRBC and interferon were added to cultures at the same time and PFC responses were determined 5 days later. (From Johnson, H. M. and Baron, S., *Cell Immunol.*, 25, 106, 1976. With permission.)

reaction.[43] When effector spleen cells were removed from mice 3 days after injection with poly rI·poly rC, the GVH reaction was enhanced in allogeneic newborn mouse recipients of these cells. On the other hand, if the effector spleen cells were removed 7 or 13 days after the injection of poly rI·poly rC, the GVH reaction was suppressed. Thus, poly rI·poly rC either enhanced or suppressed the GVH reaction, depending on the time of removal of the effector spleen cells after injection of the donor mice with poly rI·poly rC.

In a related study, con A treatment of mouse spleen cells prior to their administration to lethally irradiated allogeneic recipients resulted in 80% or greater protection of the animals against development of overt wasting disease over a 100-day period.[44] Interferon, based on its suppressor properties, is a good candidate as a possible mediator of the con A-induced protection. In another study,[45] the intensity of the GVH reaction was significantly reduced when the donor was injected with large amounts of interferon prior to removal of cells for grafting.

Related Studies

Substances produced by mitogen- and antigen-stimulated T lymphocytes and called immune suppressor factors have been described.[46,47] These substances inhibit the humoral immune response. The in vitro kinetics of the immune suppressor activity of these substances and those of interferon immune suppressor activity are similar. These immune suppressor substances have not been tested for their antiviral properties, but they may be related to antigen-induced interferon. Work needs to be done, then, to determine the precise relationships and relevances of several discoveries that appear to be tangentially related to interferon(s) and suppression of the immune response.

A Working Model

A model that has been proposed for T cell "helper" function[48] may be useful in explaining the nature of suppressor effects in the mouse involving antigen (mitogen)-type interferon. The model is based on the B cell receiving a signal from antigen-stimulated T cells in the form of a diffusible chemical mediator that is not antigen specific. The effectiveness of the mediator in helping the B cell response is dependent on the proximity of the mediator-releasing T cell to the responding B cell. The specificity of the reaction is governed by the specificity of the antigen receptor on the B and T cell; the mediator is not inherently specific for a given B cell. The model assumes that the presence of a receptor for the antigen on both the B and T cell will bring the two cells into close proximity when they bind to antigen so that the mediator, after release from the T cell, is in sufficient concentration in the microenvironment of the B cell to exert its effect. The model can be applied directly to explain how antigen-induced mediators (such as antigen-type interferon) exert their suppressor effects. The suppressor substance (interferon) is released from the appropriate antigen-stimulated T cell and inhibits the B cell antibody response to the same antigen. Thus, the kinetics of the antibody response are a reflection

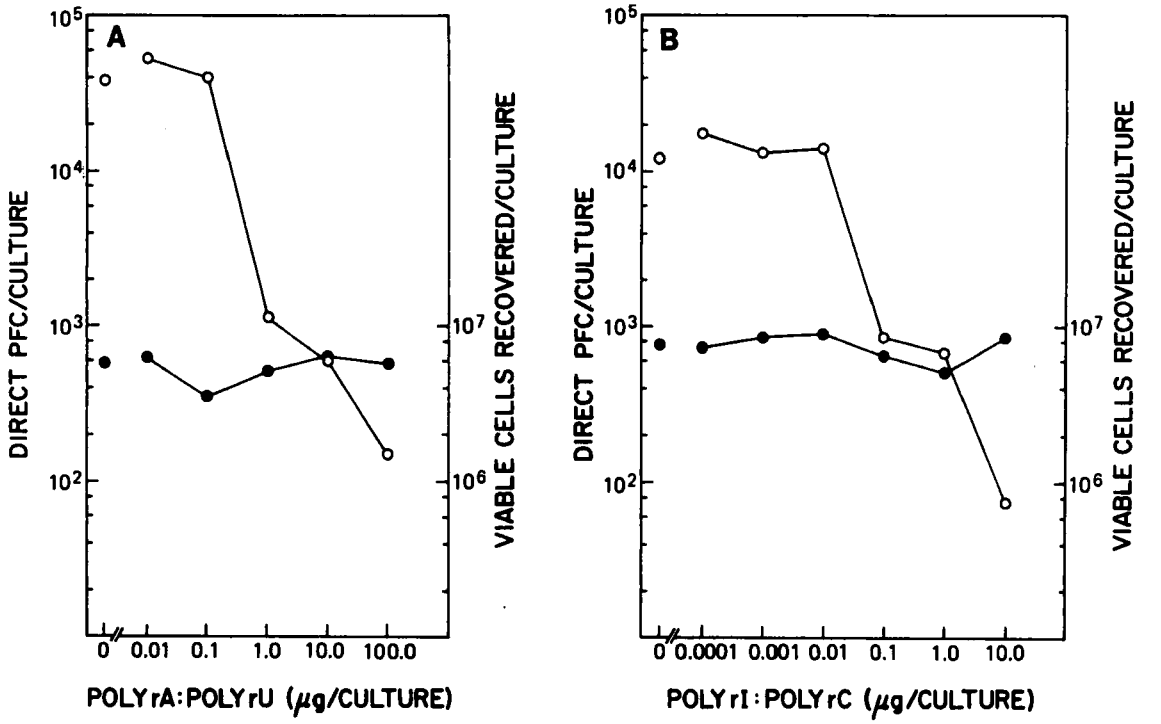


FIGURE 11. The effect of poly rA·poly rU (A) and poly rI·poly rC (B) on the primary in vitro PFC response to SRBC in C57BL/6 mouse spleen cultures. Polyribonucleotides were added at the time of SRBC addition, and direct anti-SRBC PFC per culture (o—o) and viable cells recovered per culture (•—•) were determined on day 5. (From Johnson, H. M., Bukovic, J. A., and Smith, B. G., *Proc. Soc. Exp. Biol. Med.*, 149, 599, 1975. With permission.)

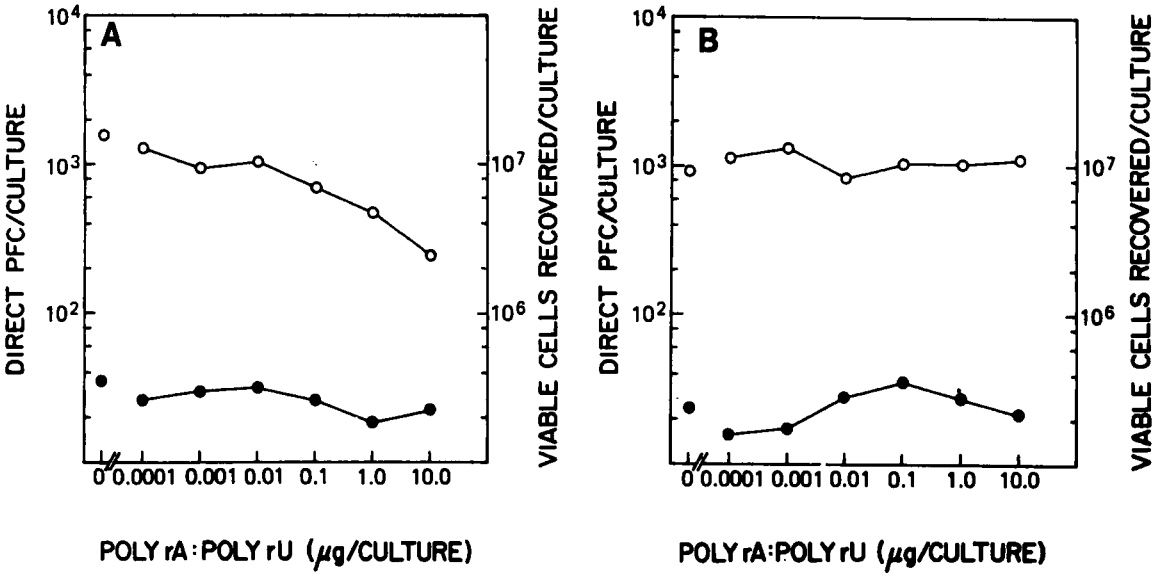


FIGURE 12. The effect of poly rA·poly rU on the primary in vitro PFC response to *E. coli* 0127 LPS in C57BL/6 (A) and nude (athymic) (B) mouse spleen cultures. The polyribonucleotide was added at the same time as antigen, and direct anti-*E. coli* 0127 LPS PFC per culture (o—o) and viable cells recovered per culture (•—•) were determined on day 5. (From Johnson, H. M., Bukovic, J. A., and Smith B. G., *Proc. Soc. Exp. Biol. Med.*, 149, 599, 1975. With permission.)

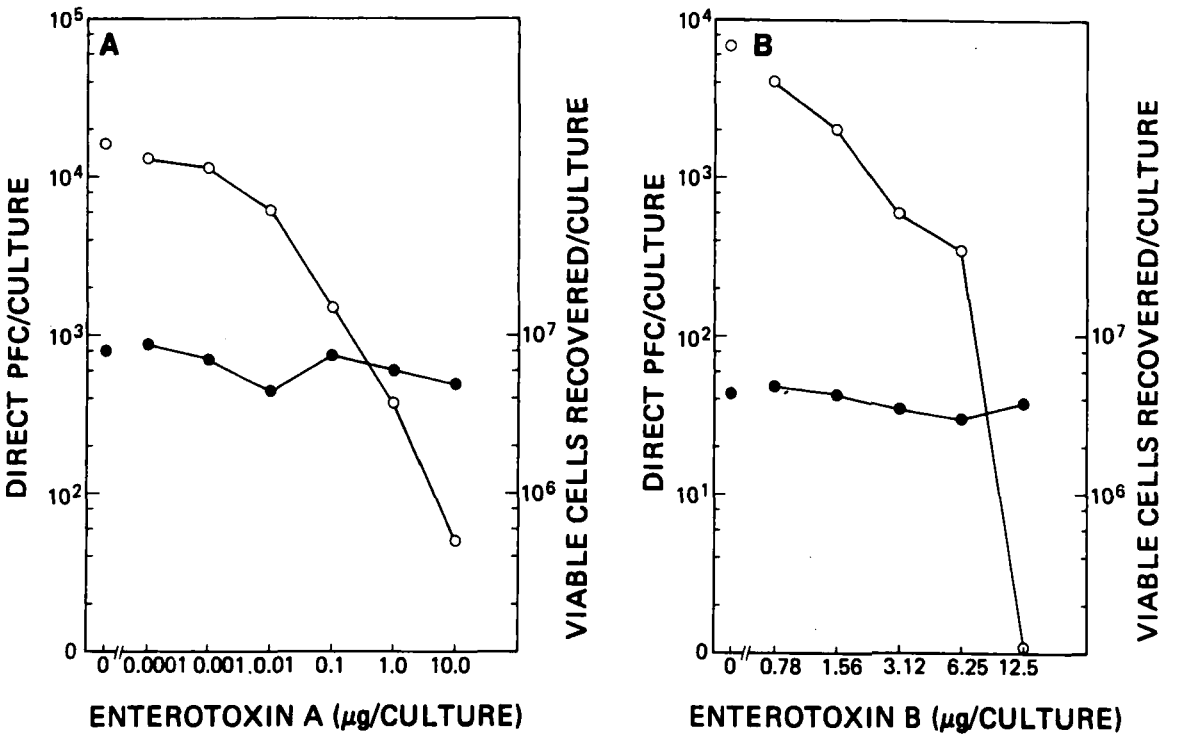


FIGURE 13. The effect of staphylococcal enterotoxins A (A) and B (B) on the primary in vitro PFC response to SRBC. Toxins were added at the time of SRBC addition, and direct anti-SRBC PFC per culture (○—○) and viable cells recovered per culture (●—●) were determined on day 5. (From Smith, B. G. and Johnson, H. M., *J. Immunol.*, 115, 575, 1975. With permission. © 1975, The Williams & Wilkins Co., Baltimore.)

of a dynamic interaction of "helper" and "suppressor" activities with the responding B cell.

The model requires modification for postulating how cells interact in suppressor effects involving virus-type interferon. Virus-type interferon is produced by virtually every cell type in the body.[1] An agent (virus, for example) that is capable of stimulating the production of virus-type interferon can theoretically shut down the immune response, provided that the interferon-producing cell produces an effective concentration of interferon in the microenvironment of the responding B cell. Viruses with infectivities that are intimately associated with the lymphoid tissues may have, in general, a greater suppressive effect on the immune response than viruses not associated with lymphoid tissue. Because of the intimate association of macrophages and T cells with the responding B cells, these cell types would be expected to play a prominent role in suppressing the immune response through virus-type interferon. As mentioned above, it has been shown that T cells are required for the virus-type interferon inducers poly rA·poly rU and poly rI·poly rC to inhibit the in vitro PFC response to *E. coli* 0127 LPS.[25] We have shown above that this inhibition is blocked by antiserum to virus-type interferon. Given this broadly outlined model, more work still needs to be done in order to precisely determine the role of various types of interferons in the regulation of the immune response. It remains to be determined whether other reported suppressions[46,47] are related to interferon. This working model provides a guide for determining the role of interferon in the mechanism of regulation of the immune response.

Finally, evidence strongly suggests that virus-type interferon inhibits the growth of a wide range of DNA and RNA viruses by blocking the translation of viral messenger RNA.[49-51] The data on the mechanism of action of interferon at the molecular level are potentially quite useful in helping to elucidate the biochemical mechanism of action of suppressor T cell effects.

MECHANISMS GOVERNING THE CELLULAR RESPONSE TO INTERFERON LEADING TO PRODUCTION OF THE ANTIVIRAL PROTEIN

Cellular Site of Action of Interferon

Originally, it was believed that interferon acted intracellularly to induce the antiviral state. This interpretation was based on the early observations that applied interferon was rapidly adsorbed by many cells in culture.[52,53] Subsequent studies indicated that it is not necessary for interferon to be consumed or irreversibly adsorbed in detectable amounts by cells to render them resistant to viral infection. Specifically, cultures of chicken or mouse cells did not detectably adsorb interferon from the culture medium during the time it took the interferon to induce antiviral activity in the cells, and no detectable uptake of interferon occurred during eight serial transfers of chicken interferon on chicken embryo cell cultures.[54]

Further evidence for the lack of detectable consumption of interferon came from the finding that the magnitude of antiviral activity induced by interferon depended on the concentration of interferon in the extracellular fluid, rather than on the total amount of interferon in the culture fluid.[55] If interferon was consumed in the process of inducing resistance, then the degree of resistance would be proportional to the total amount of interferon added rather than the concentration of interferon present.

These findings raised the possibilities that interferon was not consumed during induction of the antiviral state and, therefore, did not necessarily activate the cell from an intracellular site. The dependency on concentration for the magnitude of the interferon effect, the lack of detectable uptake of interferon by cells, plus the observation that interferon was not detectable intracellularly[56-61] suggested that either undetectable quantities of interferon penetrate cells through a limited number of sites or that interferon was acting directly at sites on the plasma membrane.

Direct evidence to support the view that interferon acts on the surface membranes of cells has come from studies utilizing interferon covalently bound to Sepharose.[61,62] Mouse interferon bound to Sepharose was incapable of penetrating the cell membrane, but was able to induce the antiviral state in mouse cells. Appropriate control experiments demonstrated that elution of interferon from the Sepharose did not quantitatively account for the degree of induction of antiviral activity. Other experiments showed that repeated cell to cell transfer of interferon bound to Sepharose did not result in loss of interferon activity.[54,62,63] Since interferon bound to relatively few Sepharose beads (one Sepahrose bead per 400 cells) rolling in cultures was able to induce the antiviral state, it appears that reversible binding of interferon to cell membranes can result in the development of the antiviral state. Although these data do not entirely exclude the possibility that a few interferon molecules might be detached from beads in contact with the cell membrane and react internally within cells, the most plausible explanation is that the Sepharose-bound interferon induced the antiviral activity by reacting with a membrane site on the cell.[62,63]

A question related to the external site of action of interferon on the membrane is whether interferon induces the antiviral state within the same cell that produced it by some internal action, or if the interferon first must be externalized and then act at the membrane surface. Two studies indicate that newly produced interferon must first be externalized in order to react with the cell membrane to induce antiviral acitivty within the interferon-producing cell. In one study, ouabain was used to treat BSC-1 monkey cells in the presence of added interferon.[64] Ouabain is known to inhibit membrane-associated adenosine triphosphatase and causes cell swelling due to an imbalance of salt and water exchange, but does not affect cellular RNA or protein synthesis at the concentration used. Ouabain treatment of interferon-producing BSC-1 cells did not inhibit interferon production, but did inhibit the development of the antiviral activity, suggesting that externalization of intracellularly induced interferon is necessary before the antiviral state can be established. In contrast to monkey cells, ouabain did not inhibit interferon action on mouse cells where it does not affect membrane adenosine phosphatase in the concentrations used. The authors suggested a surface membrane site on cells for the action of externally applied interferon. Secondary effects cannot entirely be excluded in this type of system. Additional evidence that interferon has to be released by cells before inducing the antiviral state has come from studies using antibodies to interferon to treat interferon-

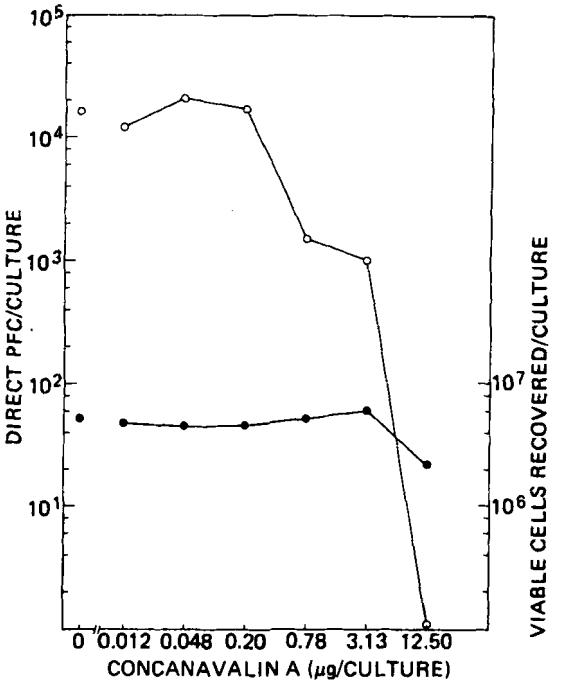


FIGURE 14. The effect of concanavalin A on the primary in vitro PFC response to SRBC. The mitogen was added at the time of SRBC addition, and direct anti-SRBC PFC per culture (○—○) and viable cells recovered per culture (●—●) were determined on day 5. (From Smith, B. G. and Johnson, H. M., *J. Immunol.*, 115, 575, 1975. With permission. © 1975, The Williams & Wilkins Co., Baltimore.)

producing cells.[65] Human fibroblast cells in culture were stimulated with a double-stranded synthetic polyribonucleotide (poly rI·poly rC) to produce interferon. When anti-interferon was added to the medium of the cells in which interferon synthesis had been induced by poly rI·poly rC, the antiviral state did not develop. Appropriate control experiments diminished the possibilities that (a) the result was due to nonspecific effect of antibody directed against cell membrane and that (b) only a small fraction of the induced cells was producing interferon, which was then inhibited by the anti-interferon before it reached nonproducer cells. These findings indicate that interferon is probably externalized from the interferon-producing cell and reacts with the cell membrane to induce the antiviral state. Further, they are consistent with the interpretation that the interferon-producing cells must first produce interferon before producing the antiviral protein for development of the antiviral state.[66]

In summary, the available evidence supports the view that interferon reacts with the cell membrane to induce the antiviral state. Interferon is apparently not consumed in this process. The action of newly produced interferon to induce antiviral activity within the producing cell occurs only after its externalization.

Cellular Receptors for Interferon

An external cellular site for the action of interferon in inducing the antiviral protein is strongly indicated by the above findings. Studies are beginning to probe and elucidate the nature of the characteristics of the proposed surface membrane site for the action of virus-type interferon. Binding of interferon to human and mouse cells at 37°C reaches a maximum between 15 and 30 min following exposure to interferon.[59,60,67] The amount of interferon bound to cells was between 1% and 4% of the interferon applied.[56-60,67] Figure 15 illustrates the kinetics of the binding of mouse interferon to mouse L cells at 37°C or 4°C. The bound interferon eluted from human or mouse cells at 37°C within 30 to 60 min after removal of unbound interferon.[59-61,67] Figure 16 illustrates the kinetics of dissociation of cell-bound interferon from mouse L cells. The location of the bound interferon appears to be on the cell surface in most cases because it is accessible to inactivation by trypsin or antibody to interferon.[58,60,61,68] With one strain of mouse cells, the interferon bound to (or associated with) cells at 37°C was inaccessible to the action of trypsin, but it was elutable from cells by further incubation.[67]

It remains to be determined whether the bulk of the cell-bound interferon is specifically bound to sites which are responsible for induction of the antiviral protein, or whether the major portion of the interferon is bound nonspecifically and only a small fraction is actually bound to receptors that result in induction of the antiviral state. Evidence favoring the specificity of binding of interferon is the fact that the amount of interferon bound to various cells can be correlated with the level of antiviral activity induced in those cells.[59,67,69]

Evidence suggesting that the bulk of the cell-bound interferon may be nonspecifically bound comes from several areas. An initial report that there was poor correlation between the amount of interferon bound to various cells and the level of antiviral activity[60] was modified to indicate that the correlation was generally positive, but there were serious exceptions.[70] For example, hamster

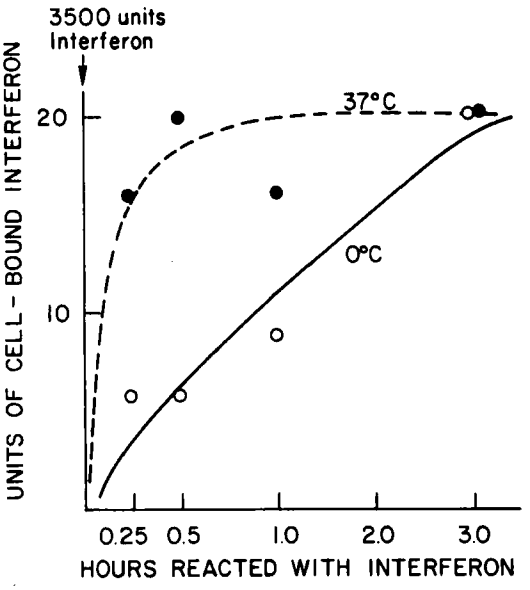


FIGURE 15. Kinetics of binding of mouse interferon to mouse L cells at 37°C or 4°C. (From Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., Studies of the role of cell-bound interferon in the induction of antiviral activity, in *Effects of Interferon on Cells, Viruses and the Immune System*, Geraldes, A., Ed., Academic Press, New York, 1975. With permission.)

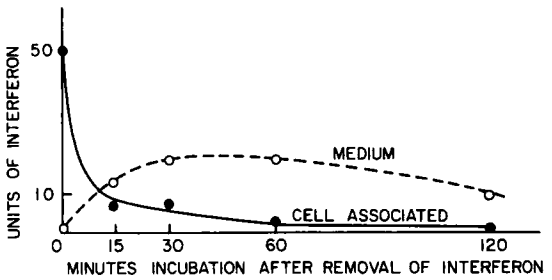


FIGURE 16. Kinetics of dissociation of cell-bound interferon from mouse L cells. Cells were exposed to 5,000 units/ml of interferon for 1 hr at 37°C and then washed six times and refed. Subsequently, interferon levels in cells and fluid were determined at intervals. (From Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., Studies of the role of cell-bound interferon in the induction of antiviral activity , in *Effects of Interferon on Cells, Viruses and the Immune System*, Geraldes, A., Ed., Academic Press, New York, 1975. With permission.)

BHK 21-7M-1 cells bound as much mouse interferon as did mouse L cells, but the hamster cells did not develop antiviral activity. Secondly, it has been observed that several proteins, such as those derived from cells and bovine albumin, may bind to cells.[54,61] Thus, some cells appear to bind many proteins and possibly portions of added interferon nonspecifically. Therefore, the bound interferon may not have induced the antiviral state in the cell due to the original binding, but may have induced the major portion of antiviral activity after subsequent elution into the extracellular fluid and additional reaction with the same cells, implying the possibility of a brief, specific reaction of induction with cells.[60] For example, the concentration of eluted interferon in the culture medium was quantitatively sufficient to account for the level of resistance which was eventually induced in washed cultures. Also, repeated washings during the elution period (to remove eluted interferon) inhibited the development of antiviral activity in mouse L cells (Figure 17). Similarly, continuous replacement of the culture medium during the elution period inhibited the development of antiviral activity (Figure 18), and increasing the volume of culture medium during and after elution proportionately inhibited antiviral activity (Figure 19).[61] Thus, antiviral activity seems to be determined mainly by a continuing reaction of interferon in the extracellular fluid, with the cell surface.

Comparison of interferon receptor activity (as measured by interferon activity) of monkey with mouse cells showed that trypsinization of the monkey, but not mouse cells, inhibited the action of the homologous interferon.[71,72] This was interpreted to indicate that the receptor for interferon on monkey cells contained a trypsin-sensitive polypeptide, but secondary effects of the trypsinization could not be completely excluded. Synergistic activity of monkey and mouse interferons reacting with a monkey-mouse hybrid cell was interpreted to suggest two distinct types of receptors on these hybrid cells.[73]

Additional evidence for interferon receptor activity on human cells comes from experiments which show that antibodies directed against human cellular antigens coded for by human chromosome 21 (which is required for interferon induction of antiviral activity) prevent the induction of antiviral activity by human interferon.[74] This finding was interpreted to indicate that the cell receptor for interferon was coded by chromosome 21. Surprisingly, the antibody could be added to the media for up to 3 hr after the interferon and still inhibit the development of antiviral activity. Since antiviral activity is strongly developed in most cell systems by 3 hr of

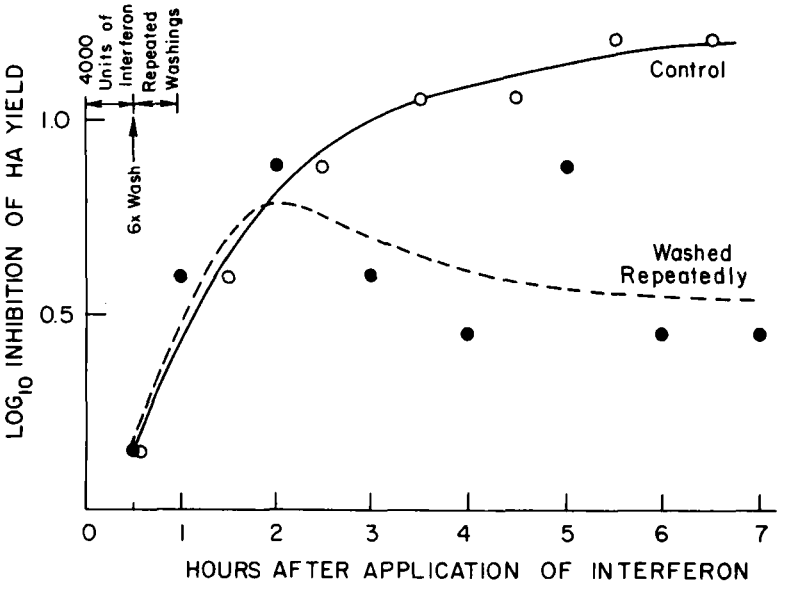


FIGURE 17. Effect of repeated washing during the disassociation period on the development of antiviral activity in mouse L cells. (From Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., Studies of the role of cell-bound interferon in the induction of antiviral activity, in *Effects of Interferon on Cells, Viruses and the Immune System*, Geraldes, A., Ed., Academic Press, New York, 1975. With permission.)

incubation with interferon, the possibility of secondary effects unrelated to the interferon receptor must be considered.

The possibility that certain gangliosides may serve as cellular receptors for mouse and human interferon was suggested by recent studies.[75,76] Addition of various gangliosides and their oligosaccharide-containing subcomponents to culture medium containing mouse interferon inhibited the antiviral action of interferon. GM2, but not GM3, inhibited interferon action in the mouse system, suggesting that a terminal *N*-acetyl galactosaminyl residue might be important. The findings that neither sialic acid nor lactose alone could reverse the inhibition of interferon by gangliosides, whereas a mixture of both was effective to some extent, supports the interpretation that the binding site for interferon includes both sugar residues. A survey of a variety of glycoproteins with possible inhibitory action on the antiviral activity of interferon indicated that the required structure is related to sialyl-galactosyl residues, which comprise a major part of the oligosaccharide structure of GM2. Studies in human cells indicated that cells with decreased amounts of certain gangliosides responded more poorly to interferon than did cells with normal amounts.[76] Treatment of these cells with gangliosides increased the ganglioside content of the cells and also the sensitivity of the cells to the action of interferon. Other cell types which failed to take up the added gangliosides failed to show an increase in interferon sensitivity. In mouse cells, GM2, GT1, and a mixture of crude gangliosides were effective, whereas GM1 and GD1A were without effect. Consistent with the interpretation was the finding that the sensitivity to interferon of one GM1-deficient, transformed, mouse cell line was not increased by pretreatment with the ganglioside.

Interferon and Derepression of the Gene for the Antiviral Protein

It seems probable from the data described above that interferon reacts with the surface of cells. A signal generated by this interaction may be conveyed from the cell surface to the appropriate gene site to induce the transcription of the mRNA for the antiviral protein. Studies in a human fibroblast cell system indicate that the time between interaction of interferon with the cell surface and transcription of the mRNA for the antiviral protein is between 30 and 45 min.[77] In these studies, interferon (at high concentration)

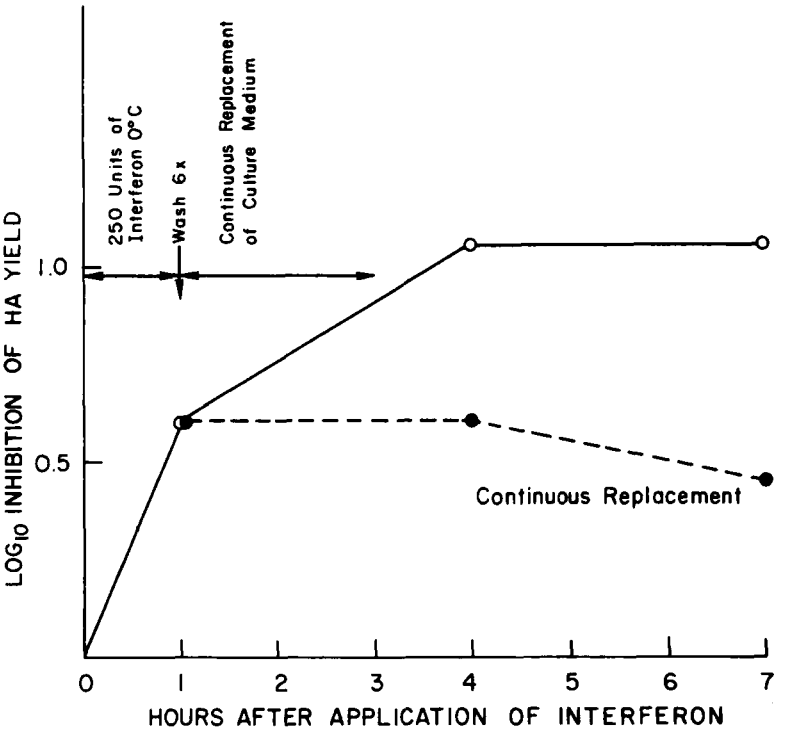


FIGURE 18. Effect of continuous replacement of culture medium during the dissociation period on the development of antiviral activity. (From Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., Studies of the role of cell-bound interferon in the induction of antiviral activity, in *Effects of Interferon on Cells, Viruses and the Immune System*, Geraldes, A., Ed., Academic Press, New York, 1975. With permission.)

was reacted with cells for just a few minutes and removed by washing, and the cells were treated with anti-interferon, with subsequent induction of antiviral activity. Timed addition of actinomycin D to cells that had reacted briefly with interferon indicated that the first mRNA was transcribed between 30 and 45 min. Thus, 30 to 45 min. are required for the reaction of interferon with the cell surface to result in transcription. The mechanism of transmission of the effect from the cell membrane to the nucleus may be similar to that of certain hormones on cells, i.e., migration of a surface membrane protein to the chromatin.[72,78,79]

Chromosomal Localization of the Genes Controlling the Interferon System

Clones of hybrid cell lines (monkey-mouse and mouse-human cell hybrids) have been used to help identify the chromosomal location of genes of monkeys and humans associated with interferon production and with production of the antiviral protein. Karyotypic analysis of four hybrid clones and the parental cell line of a somatic monkey-mouse hybrid cell line enabled the localization of the chromosomes governing the interferon system in monkey cells.[80] The genetic site governing the synthesis of monkey interferon was located on a small subtelocentric monkey chromosome. The genetic site responsible for the synthesis of the antiviral protein appeared to be located on a different (a very small subtelocentric) chromosome.[72,81]

Similar studies using human-mouse somatic cell hybrids have provided evidence that the genetic factor that codes for the antiviral protein is located on human chromosome 21.[82] Genetic control of production of human interferon was assigned to two human chromosomes, chromosomes 2 and 5.[83] Three possible explanations were offered for the requirement of two human chromosomes for human interferon production. First, one of the two chromosomes may contain a genetic factor that codes for a specific receptor site necessary for the processing of interferon inducers into signals that activate the structural

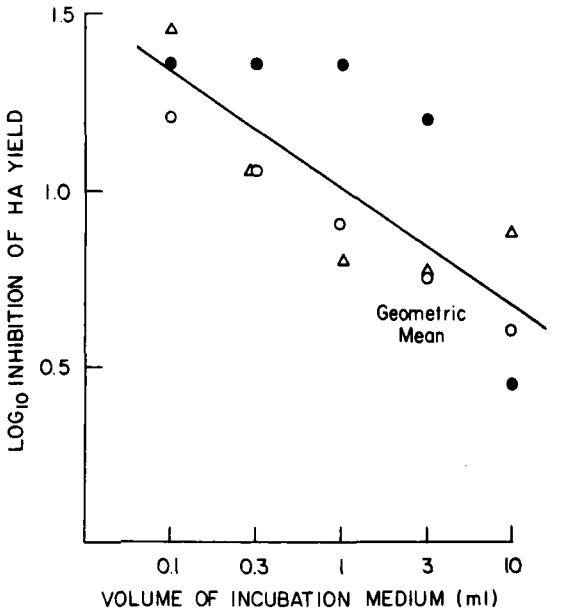


FIGURE 19. Effect of volume of culture medium during and after the disassociation period on the development of antiviral activity. (From Kohno, S., Buckler, C. E., Levy, H. B., and Baron, S., Studies of the role of cell-bound interferon in the induction of antiviral activity, in *Effects of Interferon on Cells, Viruses, and the Immune System*, Geraldes, A., Ed., Academic Press, New York, 1975. With permission.)

gene for interferon production, and the other human chromosome may contain the structural gene for interferon. Second, both chromosomes may contain genes that code for interferon subunits. Third, one chromosome may contain a structural gene for a precursor interferon and the other chromosome may contain a gene for a factor involved in the processing of the precursor to an active form.[84] As would be predicted, increased responsiveness to the antiviral action of interferon occurs in cells trisomic for chromosome 21.[85-87] Studies designed to test the hypothesis that any increase in the numbers of chromosome 21 per cell should be followed by a linear and proportional increase in the amount of the directed gene product (antiviral protein) unexpectedly showed a logarithmic increase in antiviral activity rather than an arithmetic increase.[86] This finding indicated that the genetic control is complex. This complexity is underscored by the finding that hybrid clones which contain only the human G-21 chromosome, but no other human chromosome, cannot develop the antiviral state after treatment with primate interferon.[72] The presence of any other chromosome, in addition to G-21, reestablishes the antiviral response to primate interferon. This has been interpreted to indicate that additional human chromosomes are necessary to aid chromosome 21 in specification of the cellular receptors for interferon. (Cellular receptors for interferon are considered elsewhere.)

The finding that human cells trisomic for chromosome G-21 had increased responsiveness to interferon led to a study of the responsiveness of these trisomic cells to induction of the non-antiviral activities of interferon.[88] As background, interferon may induce several non-antiviral activities in cells including: (a) toxicity in the presence of double-stranded RNA; (b) priming of cells for subsequently enhanced production of interferon; (c) increased phagocytosis by macrophages; and (d) inhibition of immunocompetent cells.[88] It was found that only the antiviral activity of interferon, and not the non-antiviral activities (cell "toxicity" and priming action, etc.), was enhanced in trisomic cells. If interferon can be assumed to react with only one specific surface receptor on cells, then these findings would not be consistent with the interpretation that chromosome G-21 codes for cellular receptors for interferon because these cells also should have enhanced non-antiviral activities if their receptors were increased. At least these findings indicate the complexity of the interferon and antiviral protein genetic control mechanisms.

Regulation of Production of the Antiviral Protein(s)

The two known proteins of the interferon system, interferon and the antiviral protein, are influenced and regulated by several factors. Production of interferon requires an inducing substance (virus, nucleic acid, or mitogen).[40,41,90] Regulation of the ongoing production of interferon is thought to be mediated by a repressor protein in certain cell species. The hypothesized regulator is newly produced by cells during production of interferon.[91-94]

Regulation of the production of the second protein, the antiviral protein, is determined by the extracellular concentration of the inducer (interferon) and perhaps by a cellular regulator. The interpretation that the level of intracellular antiviral protein is regulated in part by the concentration of interferon in the extracellular fluid, which is impinging on the cell, is based on a series of observations. As considered above, interferon appears to stimulate cells by reacting with a cellular membrane receptor. Also considered

previously is the finding that the concentration of interferon in the extracellular fluid determines the level of antiviral activity ultimately developed by a cell. Figure 20 shows the kinetics of development of resistance to multiplication of vesicular stomatitis virus by cultures of mouse embryo cells treated with varying amounts of interferon.[55] As may be seen, reaction of the cells with interferon resulted in rapidly increasing antiviral activity over about 7 hr. Thereafter, antiviral activity remained relatively stable as long as the same concentrations of interferon were reacting with the cells. The final level of antiviral activity was proportional to the concentration of interferon applied. Figure 21 shows the effect of removing interferon or increasing the concentration of interferon in mouse embryo cultures pretreated with interferon for 24 hr. As may be seen, alteration of the extracellular concentration of interferon changed the level of antiviral resistance (antiviral protein). Thus, increasing the interferon concentration from 5 units/ml to 50 units/ml resulted in increased antiviral activity which was equal to that of cultures which had not been pretreated with interferon but which were exposed to 50 units/ml of interferon for 6 hr. Alternately, removal of

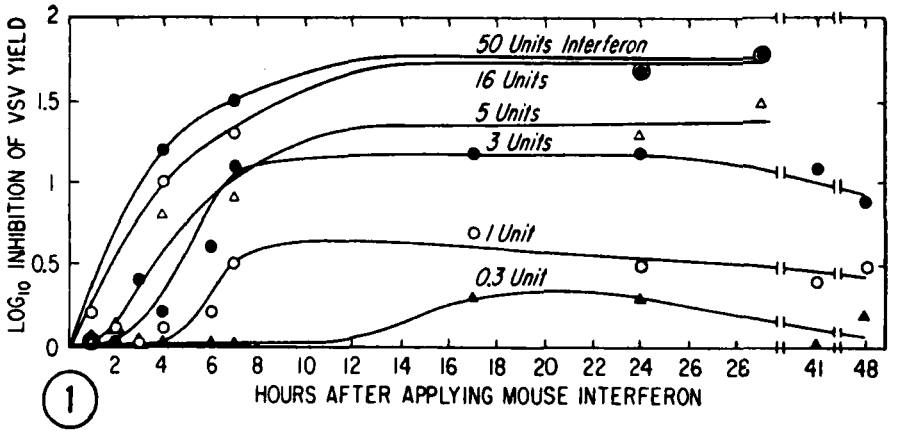


FIGURE 20. Time course of development of resistance to multiplication of vesicular stomatitis virus by cultures of mouse embryo cells treated with varying amounts of interferon. (From Baron, S., Buckler, C. E., Levy, H. B., and Friedman, R. M., *Proc. Soc. Exp. Biol. Med.*, 125, 1320, 1967. With permission.)

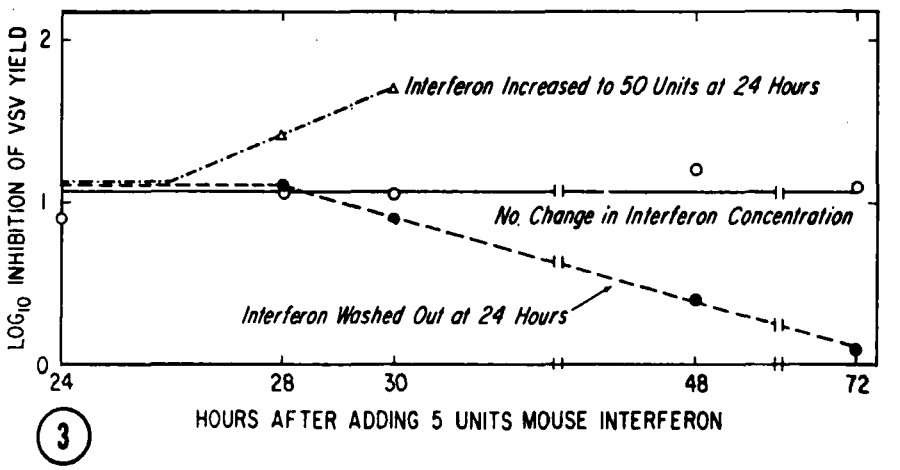


FIGURE 21. Effect of changing concentration of interferon preexisting level of resistance to vesicular stomatitis virus by mouse embryo cultures pretreated with interferon. (From Baron, S., Buckler, C. E., Levy, H. B., and Friedman, R. M., *Proc. Soc. Exp. Biol. Med.*, 125, 1320, 1967. With permission.)

extracellular interferon resulted in a declining level of antiviral activity which began to decline 7 hr after removal of interferon. Thus, the level of antiviral protein seems to be regulated, at least in part, by the extracellular concentration of interferon. Related studies involving the measurement of production of messenger RNA for the antiviral protein[55,95] indicate that the concentration of extracellular interferon regulates the level of antiviral protein by affecting the rate of transcription of the messenger RNA for the antiviral protein.

Whether there is a regulatory substance for the antiviral protein produced in cells during the development of the antiviral state cannot be finally determined from the available information. As indicated above, cells which are producing antiviral protein in response to interferon in the culture medium respond normally to increased amounts of interferon, indicating the apparent absence of a regulatory substance. The same results have been obtained in several different laboratories.[55,96,97]

Other studies have indicated the possibility that a regulator of the antiviral protein may be produced within cells responding to interferon. In experiments where actinomycin D was added to mouse L cells which had reacted with interferon for 4 to 18 hr, and the cells subsequently incubated for an additional 18 hr before virus challenge, there was potentiation of the antiviral activity of interferon against vesicular stomatitis virus.[72,98-100] These experiments are highly complex in that: (a) the actinomycin D dose varied from 0.05 μg/ml to 1 μg/ml; (b) although 1 μg/ml of the actinomycin D blocked 80% of RNA synthesis in the L cells, there was unexpectedly no significant drug toxicity over the approximately 28-hr exposure to actinomycin D;[99] and (c) the use of an 18 hr incubation time after addition of actinomycin D and before virus challenge could allow for differential decay of the antiviral protein in actinomycin D-treated as compared with untreated cells. In related experiments where actinomycin D was added after 3 and 6 hr of incubation of cells with interferon, no potentiation of the antiviral activity of interferon was observed[95] (Figure 22). It may be that the actinomycin D affected the repressor mechanism

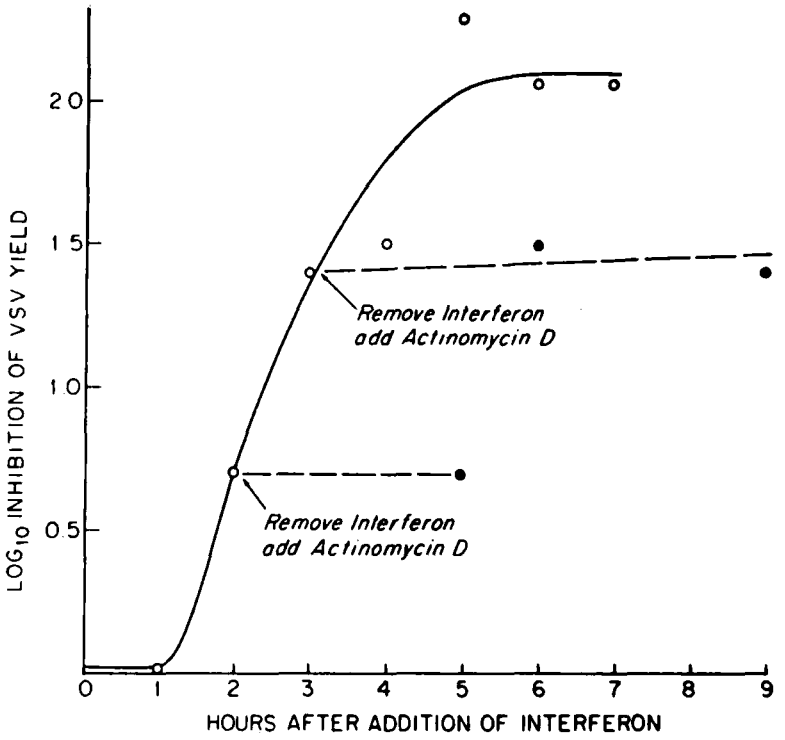


FIGURE 22. Effect of actinomycin D added with challenge virus on the development of resistance in mouse embryo cells treated with interferon. (From Dianzani, F., Buckler, C. E., and Baron, S., *Proc. Soc. Exp. Biol. Med.*, 129, 535, 1968. With permission.)

only under the conditions used in the more complex studies.

Several laboratories have described tissue and cell extracts which antagonize the antiviral action of interferon.[72,98] It has been suggested that some of the tissue antagonists might be identical with the hypothesized regulator of the antiviral protein.[98]

Summary of Cellular Events Leading to Production of the Antiviral State

Virus-type or virus-induced interferon, then, appears to exert its effects on the cell by reacting with a receptor(s) at the cell surface. Gangliosides, or more specifically, the sugar moieties of certain gangliosides may serve as the receptors for interferon. The relationship between the binding of interferon to the gangliosides at the cell surface and the subsequent events leading to the production of the antiviral protein remains to be determined. The nature of the events occurring following the binding of virus-type interferon to the cell surface that results in the transcription of the messenger RNA for the antiviral protein is not known. It is known that in the human fibroblast cell system, the messenger RNA for the antiviral protein appears between 30 and 45 min after binding of interferon to the cell surface. The genetic control of the production of human interferon resides on chromosomes 2 and 5, while the genetic factor that codes for the antiviral protein is located on chromosome 21. Studies with various cell hybrids show that the actual genetic control of the production of the antiviral protein is complex and is not a simple derepression of the appropriate gene site on the chromosomes mentioned above. The regulation of the production of the antiviral protein is determined by the extracellular concentration of interferon and, possibly, an intracellular antiviral protein regulator factor. The current evidence (see References 2 and 3) suggests that the antiviral protein inhibits virus replication by blocking the translation of viral messenger RNA.

It would be of considerable interest to ascertain if the above events that have been determined for virus-type interferon also hold for antigen (mitogen)-type interferon. Finally, if interferons prove to play an important regulatory role in the immune response, as has been suggested by the first half of this presentation, then the vast amount of mechanistic data gathered in virus studies could be of considerable value in understanding the biochemical basis of the regulation of the immune response.

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