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Antibody and Interferon Act Synergistically To Inhibit Enterovirus, Adenovirus, and Herpes Simplex Virus Infection

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The possibility that interferon (IFN) and antibody could act together to reduce virus yields in infected cells was suggested by the simultaneous occurrence of IFN and antibody at infected sites. In the present in vitro studies, mixtures of IFNs and antibody acted synergistically to reduce virus yields in cultures infected with coxsackievirus A24, enterovirus 70, adenovirus, and herpes simplex virus. This synergistic reduction was observed in different cells and at different concentration of IFN and antibody. Although IFN and antibody acted synergistically against all viruses tested, the degree of synergism was dependent on the type of IFN or the virus. For example, IFN-β and antibody was 10 to 200 times more effective than IFN-γ and antibody against coxsackievirus A24, enterovirus 70, and adenovirus. A combination of antibody and IFN-γ was three to five times more effective than IFN-β and antibody against herpes simplex virus. In addition, we found that endogenously produced IFN-β could act synergistically with antibody to coxsackievirus A24 to increase the overall antiviral effect by $10^{5.0}$-fold. No effect of endogenous IFN was observed in herpes simplex virus-infected cultures treated with antibody to herpes simplex virus. These studies indicate the potential importance of the synergistic effect of locally produced IFN and antibody in restricting virus early in the natural infectious process. They also suggest that combinations of IFNs and antibody may be more beneficial in the treatment of certain virus infections than IFN or antibody alone.

We have recently reported the presence of fibroblast interferon (IFN-β) and a virus-specific neutralizing activity in tears of patients with conjunctivitis caused by coxsackievirus A24 (CA24) (4, 6) and enterovirus 70 (EV70) (unpublished observation). We also reported the presence of IFN-β in cerebral spinal fluid during enteroviral encephalitis and have shown that passive administration of specific antibody significantly reduced levels of recoverable virus in cerebral spinal fluid (12). The simultaneous presence of interferon (IFN) and antibody during these infections suggested that IFN and antibody could be acting together to reduce virus production in vivo. We addressed this possibility by comparing the yields of virus produced in infected cultures treated with different concentrations of antibody alone, IFN alone, or with combinations of each.

MATERIALS AND METHODS

Viruses. EV70 (SEC146/71) and CA24 (SEC24/70) isolates were obtained from M. Yin-Murphy (Department of Bacteriology, University of Singapore, Singapore) (13). Adenovirus type 3 (Ad3) and herpes simplex virus (HSV, strain F) were obtained from S. Baron (Department of Microbiology, University of Texas Medical Branch, Galveston).

Human IFNs. Human IFN-α (10^6 U/mg of protein) was a gift from K. Cantell (Public Health Center, Helsinki, Finland), and IFN-β ($10^{5.5}$ U/mg of protein) was obtained through the Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Human IFN-γ ($10^{4.7}$ U/mg of protein) was prepared with staphylococcal enterotoxin A as previously described (3).

Cell culture and diluent. Human amnionic WISH cells (Flow Laboratories, Inc., Rockville, Md.), Chang's human conjunctival cells (a gift from W. J. O'Brien, The Medical College of Wisconsin, Milwaukee), and rabbit kidney (RK_{13}) cells (Flow Laboratories) were used for growing virus stocks, assaying virus, and determining the combined effects of antibody and IFN. Eagle minimum essential medium containing Earle salts supplemented with 2% fetal bovine serum and antibiotics (EMEM) was used as the diluent for virus and IFN.

Virus quantification. Plaque assays of CA24, EV70, and Ad3 were performed in Chang conjunctival or WISH cell cultures prepared in 96-well microtiter plates (Costar, Cambridge, Mass.) (11). HSV was plaque assayed in RK_{13} cell cultures.

IFN quantification. UV-irradiated culture medium harvested from infected cultures and standard IFN preparation were assayed for antiviral activity as described previously (5, 11). One unit of IFN activity is the amount of IFN required to inhibit 50% of the cytopathogenic effect of the designated virus.

Antibodies to viruses. Monkey anti-EV70 serum (10,000 U/ml) was obtained from R. Kono (National Institute of Health, Tokyo, Japan). Rabbit anti-CA24 serum (320 U/ml) was a gift from M. Yin-Murphy. Guinea pig anti-HSV serum (2,000 U/ml) was obtained from the National Institute of Allergy and Infectious Diseases. Human serum antibody (600 U/ml) against Ad3 was obtained from D. A. Weigent (Department of Microbiology, University of Texas Medical Branch, Galveston).

Antibody to IFN-β. Antibody to IFN-β was a gift from Barbara Dalton and C. A. Ogburn (Pennsylvania College of Medicine, Pittsburg) and was included in overlay medium of certain experiments to eliminate endogenously produced IFN.

Antibody quantification. The level of specific neutralizing antibody in serum was determined by using a 50% cytopathogenic reduction assay employing 100 to 200 PFU of virus (7). One unit of antibody activity is the amount required to inhibit 50% of the cytopathogenic effect of the designated virus.

Virus production in the presence of IFN and antibody. Microtiter plate cultures of human WISH cells of Chang human conjunctival cells were challenged with a low input multiplicity of infection ($10^{-4.0}$ PFU/cell) of the respective virus. After 3 h of incubation at 37°C, unadsorbed virus was removed, and the cultures were washed three times with fresh EMEM. Then various concentrations of IFN-β or IFN-γ in EMEM (0.05 ml) and 10 U of virus-specific antibody in EMEM (0.05 ml) were pipetted onto the preinfected cells. The cultures were incubated at 37°C until 100% cell death occurred in virus controls. The culture fluids were then harvested, and the levels of virus yield were determined. The levels of any residual IFN or antibody were determined by the methods described above.

Virus production in the presence of antibody alone. WISH cell cultures were infected with $10^{-4.0}$ PFU of virus per cell, incubated at 37°C for 3 h, and washed three times with fresh medium to remove unadsorbed virus. Samples (0.1 ml) of virus-specific antibody dilutions with and without antibody to IFN-β (100 U/ml) were then added to each well. (Antibody to IFN-β was added to neutralize the endogenously produced IFN-β.) The cultures were then incubated at 37°C until 100% cell lysis occurred in virus controls. Virus yields were determined as described above.

RESULTS

Synergistic reduction of virus yields by antibody and IFN against CA24. Previous observations suggested that IFN and antibody have the opportunity to act together to control viral infections (4, 6). Therefore, to determine the antiviral effectiveness of combinations of IFN and antiviral antibody in vitro, monolayer cultures of WISH cells were infected with 10^{-4} PFU of CA24 per cell and then treated with IFN alone, antibody alone, or a combination of IFN and antibody. Low levels of IFN, antibody, and virus were used to approximate natural conditions. Figure 1 presents typical data obtained from three experiments. The results indicate

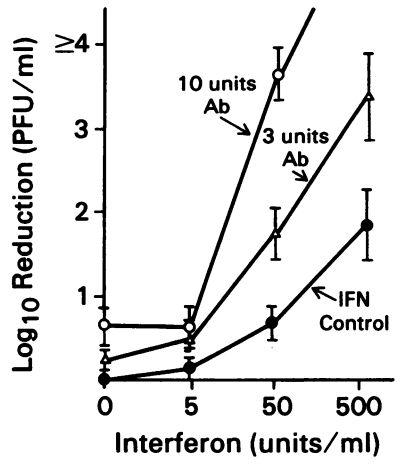

FIG. 1. Comparison of the effect of different levels of antibody and IFN-β on mean (± standard error of the mean) virus yields from human WISH cells infected with CA24 ($10^{-4.0}$ PFU per cell) in three experiments.

that 500 U of IFN-β with 10 U of antibody acted synergistically to reduce virus yields by $>10^{4.5}$ PFU (greater than 300-fold above the additive effect), whereas 500 U of IFN-β alone reduced virus yields by 1.5 $\log_{10}$ units, and 10 U of antibody alone reduced virus yields by 0.6 $\log_{10}$ units. In addition, 3 U of antibody and IFN-β (500 U) inhibited CA24 yields by 2.3 $\log_{10}$ units (1.0 $\log_{10}$ units above the additive effects).

Synergistic reduction of virus yield by antibody and different types of IFN against CA24. Since different types of IFN may be present during the various phases of a virus infection (6), we compared the virus yields from CA24-infected cells after treating with antibody to CA24 alone, IFN-α, IFN-β, or IFN-γ alone, or combinations of antibody plus each IFN. In each of three experiments, virus yields were synergistically reduced by the combination of antibody and each IFN type. Figure 2 presents typical data. The greatest reduction in virus yield ($>10^5$ PFU) was observed in CA24-infected cultures treated with 500 U of IFN-β and 10 U of antibody (Fig. 2B). Comparatively, IFN-γ and antibody was least effective, ≥10-fold lower than IFN-α and IFN-β (Fig. 2C).

Synergistic action of IFN and antibody against different viruses. In studies similar to those described above, we investigated the broadness of the synergistic effect by testing combinations of IFN and antibody against different viruses in a different cell type. Specifically, Chang human conjunctival cells, which are more sensitive to IFN than WISH cells, were preinfected with a low multiplicty of infection ($10^{-4.0}$ PFU) of

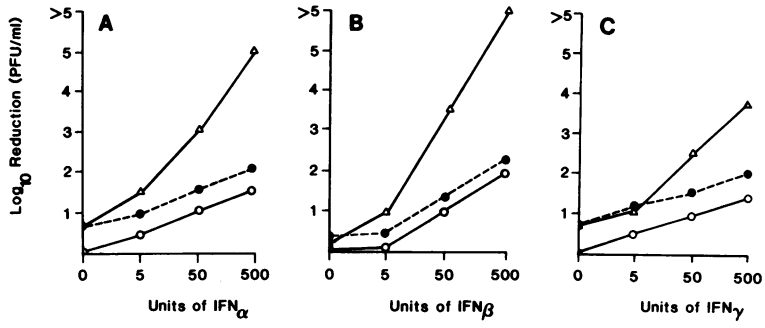

FIG. 2. Protective effect of various concentrations of IFN-α (A), IFN-β (B), and IFN-γ (C) and 10 U of anti-CA24 antibody on human WISH cells infected with CA24 ($10^{-4.0}$ PFU per cell). Shown are the amount of reduction in virus yields observed when infected cultures were treated with mixtures of IFN and 10 U of antibody (△) IFN alone (○) and the amount of virus reduction expected to occur if the independent effects were additive (●).

CA24, EV70, Ad3, or HSV, incubated for 2 h, washed twice, and treated with medium containing 10 U of virus-specific antibody, 50 U of IFN alone, or with medium containing both antibody and IFN (Fig. 3). A synergistic reduction in virus yields (5- to 80-fold) by antibody and IFN-β or IFN-γ was noted for each virus tested. Interestingly, 50 U of IFN-β plus 10 U of antibody was reproducibly more effective than 50 U of IFN-γ in reducing CA24, EV70, and Ad3 yields, whereas 50 U of IFN-γ plus 10 U of antibody was reproducibly more effective against HSV. Similar reductions were observed in WISH cells (data not shown).

Enhancement of protective effect of antibody by endogenous IFN. Because locally produced, endogenous IFN would be the most likely source of IFN early in a virus infection, we determined the ability of endogenous IFN-β and anti-CA24 antibody to protect infected cells. To accomplish this, medium containing virus-specific antibody with and without antibody to IFN-β was added to CA24- or HSV-infected cells, and the virus yields were compared. Figure 4 presents data from one of three experiments. Increasing concentrations of specific antibody decreased the virus yields in both CA24- and HSV-infected cell cultures. Interestingly, elimination of endogenously produced IFN-β by the addition of antibody to IFN-β to the overlay medium containing 1,000 U of virus-specific antibody resulted in a 10^5-fold increase in CA24 yields over that observed with 1,000 U of antibody alone. Complete abrogation of the protective effects of low levels of antibody to CA24 (<100 U) was observed when endogenous IFN-β was neutralized. In contrast, the addition of antibody to IFN-β had no effect on virus yields from HSV-infected cells treated with virus-specific antibody.

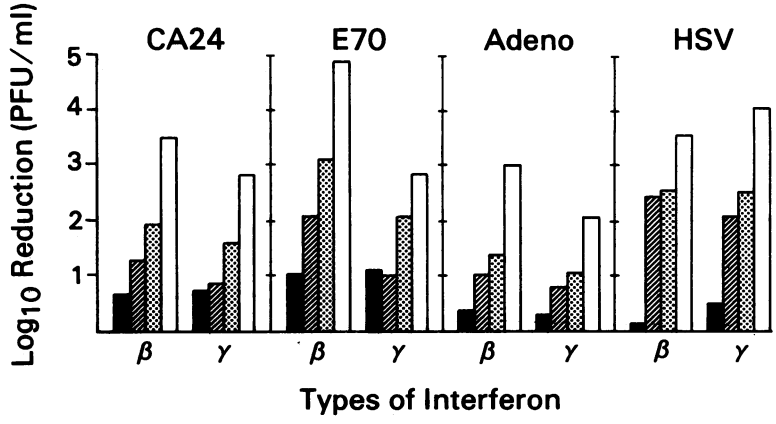

FIG. 3. Reduction of CA24, EV70, Ad3, and HSV yields in the presence of 10 U of virus-specific antibody (■) or 50 U of IFN-β or IFN-γ (▨) as compared with the expected reduction if the singular effects were simply additive (▨) and to the actual reduction observed when cultures were treated with mixtures of antibody and IFN (□).

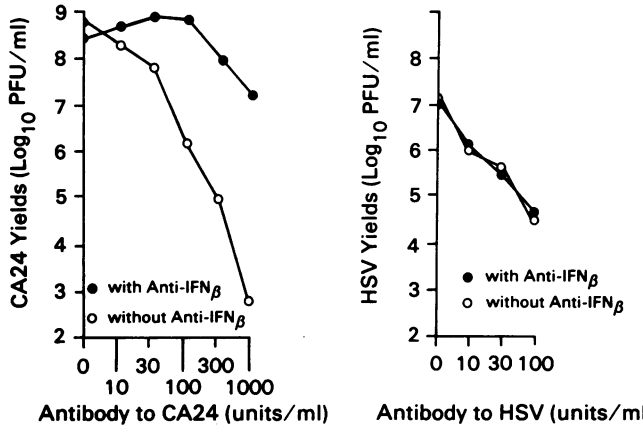

FIG. 4. Comparison of the levels of virus yields in CA24- or HSV-infected cultures after treatment with virus-specific antibody with and without antibody to IFN-β. Enough antibody to IFN-β was added to cultures to neutralize 1,000 U of antiviral activity.

DISCUSSION

The possibility that endogenous (IFNs) and an early appearing neutralizing activity produced in vivo could be acting together in a synergistic manner to help control ocular CA24 and EV70 was suggested because they occurred simultaneous during natural infection (4, 6, 11; unpublished observations). In the present studies, we examined the ability of antibody and IFN to act synergistically to inhibit CA24, EV70, Ad3, or HSV in cell culture.

We found that the addition of antibody and IFN to preinfected cultures resulted in a greater reduction (10- to >1,000-fold) of virus yields than could be accounted for if their antiviral activities were simply additive. This synergistic effect was demonstrable in different cells (human conjunctival and human amnionic WISH cells), with a variety of viruses (CA24, EV70, Ad3, HSV), with the different IFNs (α, β, and γ), and with different concentrations of antibody and IFNs. Interestingly, the synergistic action of IFN-γ and antibody was greater against HSV, whereas IFN-β and antibody were more effective against CA24, EV70, and Ad3. Also, the combination of virus-specific antibody plus IFN-α or IFN-β was more effective against CA24, EV70, echovirus 5, echovirus 33, coxsackievirus B_3, poliovirus, Ad3, Ad7, Sindbis virus, and vesicular stomatitis virus than antibody and IFN-γ against these viruses (data not shown).

The synergistic effect of combinations of antibody and IFNs is probably related to the ability of (i) antibody to retard extracellular spread of virus and to reduce the multiplicity of infection, thus allowing more time for IFN to act, and (ii) IFN to protect uninfected cells and to reduce virus yields from infected cells, thus making low levels of antibody protective. The differences in magnitude of the synergistic effects observed between different viruses and different IFNs are probably related to the ability of the antibody to neutralize the virus, the sensitivity of virus and cells to the IFN type, and the ability of virus to induce endogenous IFN. Further studies on the mechanism(s) of virus neutralization by antibody in the presence of IFN are being done.

To help determine the effect of endogenously produced IFN on the neutralizing activity of antiviral antibody, the activity of endogenous IFN was deleted in infected cultures by antibody to IFN-β. The studies showed that inhibition of the effects of endogenously produced IFN-β present in CA24-infected cultures decreased the protective effect of antibody by >99.9%. Similar results were obtained when actinomycin D was used to block IFN production in both CA24- and EV70-infected cultures (data not shown). In comparison, the addition of antibody to IFN-β to HSV-infected cells did not affect the decreases in virus yields due to antibody to HSV, suggesting that HSV did not induce IFN. Similar results were obtained with Ad3 (data not shown). The addition of IFN-α or IFN-γ to the treatment mixture containing virus-specific antibody and antibody to IFN-β synergistically inhibited HSV, EV70, and CA24 but not to the level observed when endogenous IFN was present for EV70 and CA24 (data not shown). Thus, in an in vitro cell culture system, endogenous IFN does not appear to play a role in the overall control of HSV. However, under conditions of natural infection, IFNs are produced (1, 2) that could act synergistically with antibody or other cellular components (or both) (8) to inhibit HSV. In summary, these studies indicate that the effectiveness of antibody to control virus during infection is largely dependent upon the ability of

virus to induce IFNs and the sensitivity of the virus to IFN (even low levels).

Previously, the relative importance of antibody or IFN as a significant host defense mechanism operating during the course of natural viral infection has been based upon their independent effects, mainly because of IFN deletion experiments with antibody to IFN-β (2) and because of the relative time of appearance of each during infection (10). Our findings suggest that, in some virus infections, the combined effect of antibody and IFN may be an important control mechanism for prevention of disease. Thus, combinations of even low levels of antibody and IFN which occur early during primary and secondary infection (9, 10) could be largely responsible for the rapid decrease in virus seen in many infections. Our results could explain at least part of the exacerbation of virus disease seen in IFN depletion experiments because antibody's protective effect would be expected to be significantly reduced in the presence of antibody to IFN.

This phenomenon offers another plausible explanation for the large numbers of infections that occur without manifestation of clinical disease. Concomitantly, the synergistic action of IFN and antibody may also be important factors responsible for the long-lasting immunity that follows primary infection or vaccination, especially when serum antibody levels become very low or even undetectable by standard methods.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Armstrong, R. W., M. J. Gurwith, D. Wadell, and T. C. Merigan.** 1970. Cutaneous interferon production in patients with Hodgkins disease and other cancers infected with varicella or vaccinia. N. Engl. J. Med. **283**:1182–1184.
2. **Gresser, I., M. G. Tovey, C. Maury, and M. T. Bander.** 1976. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Maloney sarcoma, vesicular stomatitis, Newcastle disease and influenza viruses. J. Exp. Med. **144**:131–1327.
3. **Langford, M. P., J. A. Georgiades, G. J. Stanton, F. Dianzani, and H. M. Johnson.** 1979. Large scale production and physicochemical characterization of human immune interferon. Infect. Immun. **26**:36–41.
4. **Langford, M. P., G. J. Stanton, J. C. Barber, and S. Baron.** 1979. Early-appearing antiviral activity in human tears during a case of picornavirus epidemic conjunctivitis. J. Infect. Dis. **139**:653–658.
5. **Langford, M. P., D. A. Weigent, G. J. Stanton, and S. Baron.** 1981. Virus plaque-reduction assay for interferon: microplaque and regular macro plaque reduction assays. Method. Enzymol. **78**:339–346.
6. **Langford, M. P., M. Yin-Murphy, Y. M. Ho, J. C. Barber, S. Baron, and G. J. Stanton.** 1980. Human fibroblast interferon in tears of patients with picornavirus epidemic conjunctivitis. Infect. Immun. **29**:995–998.
7. **Lennette, E. H.** 1969. General principles underlying laboratory diagnosis of viral and rickettsial infections, p. 43–46. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections. American Public Health Association, New York.
8. **Notkins, A. L.** 1975. Interferon as a mediator of cellular immunity in viral infections, p. 146–166. *In* Viral immunology and immunopathology. Academic Press, Inc., New York.
9. **Ogra, P., A. Morag, and M. L. Tiku.** 1975. Humoral immune responses to viral infections, p. 57–78. *In* Viral immunology and immunopathology. Academic Press, Inc., New York.
10. **Stanton, G. J., H. M. Johnson, and S. Baron.** 1978. The role of interferon in virus infections and antibody formation. Pathobiol. Ann. **8**:285–313.
11. **Stanton, G. J., M. P. Langford, and S. Baron.** 1977. Effect of interferon, elevated temperature, and cell type on replication of acute hemorrhagic conjunctivitis viruses. Infect. Immun. **18**:370–376.
12. **Weiner, L. S., J. T. Howell, M. P. Langford, G. J. Stanton, S. Baron, R. M. Goldblum, R. A. Lord, and A. S. Goldman.** 1979. Effect of specific antibodies on chronic echovirus type 5 encephalitis in a patient with hypogammaglobulinemia. J. Infect. Dis. **140**:858–863.
13. **Yin-Murphy, M.** 1973. The picornaviruses of acute hemorrhagic conjunctivitis: a comparative study. Southeast Asian J. Trop. Med. Public Health **4**:305–310.