Orally Administered Interferons Exert Their White Blood Cell Suppressive Effects via a Novel Mechanism (43499)

W. ROBERT FLEISCHMANN, JR.,[1] SRECKO KOREN,[2] AND CHRISTINA M. FLEISCHMANN
Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas 77550

Abstract. **Interferons (IFN) have been approved for a number of clinical uses. The accepted routes of administration are intramuscular, subcutaneous, and intravenous. Recently, interferons administered by the oral route have been shown to exert a systemic effect. Oral administrations of IFN-α, IFN-β, and IFN-γ have been shown to cause a suppression of the peripheral white blood cell (WBC) count in mice.This study investigates the mechanism by which this suppression occurs. The results show that, in contrast to their intraperitoneal administration, oral administration of rHuIFN-αA/D or rMuIFN-γ does not result in the presence of detectable levels of interferons in the blood. In addition, although the presence of circulating specific antibody to interferon blocks the peripheral WBC suppressive effects of intraperitoneally administered MuIFN-β or rMuIFN-γ, the presence of those antibodies does not block the peripheral WBC suppressive effects of the orally administered interferons. The peripheral WBC suppressive effect of orally administered rHuIFN-αA/D and rMuIFN-γ can be transferred by injection of blood from oral interferon-treated donor mice to recipient mice. Recipient mice receiving plasma from donor mice showed no peripheral WBC suppression. Recipient mice receiving blood cells from donor mice showed significant peripheral WBC suppression. No effect of orally administered rHuIFN-αA/D on the relative percentages of lymphocytes, neutrophils, and monocytes was noted. These results indicate that the mechanism by which orally administered interferons exert their WBC suppressive effect differs from that of intraperitoneally administered interferons. WBC suppression resulting from orally administered interferons may involve cell to cell transfer of the interferons' effects, rather than the systemic distribution of the interferons in the blood. These studies further suggest that there may be a role for oral administration as a new route of interferon administration and provide a glimpse into the mechanism by which the orally administered interferons exert their systemic effects.** [P.S.E.B.M. 1992, Vol 201]

Interferons (IFN) have recently been approved for a number of clinical applications (1). For these clinical applications, interferons are currently administered subcutaneously, intramuscularly, or intravenously. However, there are several limitations with these routes of administration. IFN-β and IFN-γ appear to be retained at the local site of injection and are not well distributed after subcutaneous or intramuscular injection (2).

A number of studies have addressed whether the oral route may serve as an effective route of interferon delivery. These studies have provided some evidence that the oral route of interferon administration may exert local (3–10) or, possibly, even systemic (11–13) antiviral effects.

Recently, the oral route of interferon administration was evaluated for its ability to exert a systemic effect, suppression of peripheral white blood cell (WBC) counts (14). Suppression of peripheral WBC counts was chosen for evaluation because it represented a rapidly occurring effect of interferon that could be readily quantitated. In this study, each of the three interferons (rHuIFN-αA/D, MuIFN-β, and rMuIFN-γ) was shown

[1] To whom requests for reprints should be addressed at Department of Microbiology, The University of Texas Medical Branch, Galveston, TX 77550.

[2] Permanent address: Institute of Microbiology, Medical Faculty, University of Ljubljana, 61105 Ljubljana, Slovenia, Yugoslavia.

Received December 30, 1991. [P.S.E.B.M. 1992, Vol 201]
Accepted June 5, 1992.

0037-9727/92/2012-0200$3.00/0

to exert a significant suppressive effect on the peripheral WBC count. However, the dose-response curves obtained with orally administered interferons were more shallow than those obtained with subcutaneously administered interferons, which suggests that the interferons administered by different routes might exert their WBC suppressive effects via different mechanisms.

The current study explores the mechanism by which orally administered interferons exert their WBC suppressive effects. We show that the mechanism by which orally administered interferons exert their WBC suppressive effect appears to be different from that of intraperitoneally administered interferon.

Materials and Methods

Mice. Pathogen-free, 6- to 8-week-old female C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). The mice were maintained in cages with autoclaved bedding. The cages were bathed in sterile air from horizontal laminar flow animal stations (Germfree Laboratories, Inc., Miami, FL). They were given autoclaved food and water *ad libitum*. Mice were monitored for exposure to mouse pathogens and were confirmed to remain pathogen-free during the course of the experiments by specific antibody testing for mouse hepatitis virus, minute virus of mice, Sendai virus, pneumonia virus of mice, GD-7, and *Mycoplasma pulmonis*.

Interferons. Recombinant DNA-derived rHuIFN-αA/D ($10^{7.8}$ units/mg protein) was kindly provided by Drs. Michael Brunda and Peter Sorter (Hoffmann-LaRoche, Nutley, NJ). Natural MuIFN-β ($10^{8.3}$ units/mg protein) was purchased from Lee Biomolecular Research Laboratories (San Diego, CA). Recombinant DNA-derived rMuIFN-γ ($10^{7.0}$ units/mg protein) was kindly provided by Dr. Paul Trotta (Schering Corp., Bloomfield, NJ). Interferon titers were determined in a microtiter plaque reduction assay (15) and compared with the appropriate NIH Reference Standards. Interferon titers are expressed as International Reference Units/ml. Interferons for oral administration were prepared in a 0.1% gelatin in water solution (gelatin/water). Interferons for intraperitoneal administration were prepared in a 3-mg/ml bovine serum albumin in phosphate-buffered saline solution (BSA/PBS).

Antibodies to Interferons. Polyclonal antibody to MuIFN-β (30,000 units/ml) was purchased from Lee Biomolecular Research Laboratories. Monoclonal antibody to MuIFN-γ (40,000 units/ml) was prepared from ascitic fluid harvested from BALB/c nude mice inoculated intraperitoneally with R4-6A2 cells (16; ATCC HB170). The monoclonal antibody to MuIFN-γ was partially purified by passage through a protein G column (ImmunoPure (G) IgG purification kit; Pierce, Rockford, IL) according to the instructions of the manufacturer. The antibody titers were determined in neutralization tests that were performed as defined by the World Health Organization. In this method, 1 unit of antibody is the amount of antibody required to reduce 10 units of interferon to 1 unit. In all but one experiment, antibodies to interferons were administered as a 0.1-ml intraperitoneal injection. In one experiment, antibodies to MuIFN-γ were administered as a tail vein injection. One day after the mice were bled for final peripheral WBC counts, the mice were exsanguinated. Their blood was allowed to clot and the serum antibody titer was determined. The peripheral WBC counts of mice that exhibited less than 30 units of antibody/ml were excluded from the experiments. Only five of 92 antibody-treated mice were excluded from the experiments. The antibody titers in the mice included in the experiments were >90 units/ml of antibody to MuIFN-β and >60 units/ml of antibody to MuIFN-γ.

Mouse Bleeding. Anesthetized mice (Ketalar, 2.5 mg/mouse; Parke-Davis, Morris Plains, NJ) were bled from the retro-orbital venous plexus. For routine bleeding experiments, 100 μl of blood were collected in a disposable Micro-pipette (Fisher Scientific Co., Pittsburgh, PA) that had been dipped in EDTA (2%; Sigma Chemical Co., St. Louis, MO) and dried. The blood was delivered to a round-bottom polypropylene tube (Falcon, 12 × 75 mm; Becton-Dickinson and Co., Lincoln Park, NJ) containing 5 μl of EDTA and thoroughly mixed. Next, the blood was diluted 1/30 in phosphate-buffered saline ([PBS] 300 μl final volume). Red blood cells were lysed by adding one drop of ZAP-OGLOBIN (Coulter Diagnostics, Hialeah, FL). Total WBC counts were made in a hemocytometer. Each count was based on the average counts of two counting chambers. Statistical analyses of the data were done using Student's *t* test. Prebleeding the day before initiation of the experiment permitted the recognition and elimination from the experiments of mice that had high and low white blood cell counts, thus giving a more narrow standard deviation.

For transfer experiments, groups of mice were treated orally with gelatin/water or with 5000 units/day of interferon in gelatin/water. One third of the donor mice were bled after 1 day of interferon treatment, another third after 2 days of interferon treatment, and the last third after 3 days of interferon treatment. On the day of bleeding, approximately 500 μl of blood were collected from each donor mouse, using capillary tubes that had been dipped in EDTA as described above. The blood from each mouse was collected in a microcentrifuge tube and centrifuged in a microcentrifuge for 3 min. In the first experiment, the plasma from each of the five mice in a group was carefully drawn off with a Pipetman pipette (P200; Rainin, Emeryville, CA), pooled (approximately 1.25 ml), and brought to a volume of 2.5 ml with PBS. The packed cells were resuspended in PBS, pooled, brought to 2.5 ml, and

counted in a hemocytometer. Groups of seven recipient mice were injected intraperitoneally with 0.3 ml/mouse of either the plasma preparation (equivalent to approximately 0.15 ml of undiluted plasma/mouse) or the cell preparation (approximately 1×10^6 cells/mouse). In the second experiment, the plasma and packed cells from each of the six mice in a group were collected and dispensed in a parallel manner. Recipient mice received treatment on three successive days, corresponding to the three days of bleeding of the donor mice.

Protocol for Oral Administration of Interferons. Mice to be used for antibody experiments that measured peripheral WBC counts were bled 1 day before initiation of the treatment. The next day, half of the mice were inoculated intraperitoneally with antibody to interferon (1000 units/mouse in 0.1 ml of BSA/PBS) and half of the mice received an equal volume of BSA/PBS. On the day after the administration of antibody, the mice were divided into groups that received oral interferon, intraperitoneal interferon, or both oral gelatin/water and intraperitoneal BSA/PBS. In these experiments, mice received oral interferon treatment in their drinking water (together with 0.1% gelatin, which was added as a stabilizer). This interferon preparation was supplied *ad libitum*, with approximately 3.5 ml consumed per mouse per day. For example, mice treated with 5000 units/day of orally administered rMuIFN-γ drank approximately 3.5 ml of a rMuIFN-γ/gelatin/water solution that was prepared to contain 1500 units/ml of rMuIFN-γ. Control mice received 0.1% gelatin in their water supply. Interferon/gelatin/water solutions were prepared before the initiation of each experiment and were stored frozen at −70°C (rHuIFN-αA/D and MuIFN-β) or −20°C (rMuIFN-γ) until needed. Storage at −70°C or −20°C had no effect on interferon titer. Freshly thawed interferon/gelatin solutions were supplied each day for the mice. After 3 days of interferon treatment, the mice were bled, WBC counts were determined, and the WBC counts were expressed for each individual mouse as the percentage of the prebleed WBC count. A day after the bleeding for WBC counts, the mice were again bled without using EDTA. The blood was allowed to clot and the cells were spun out of the serum using a microcentrifuge (Fisher Scientific). The serum was titered for antibody as described above.

Mice used for determinations of interferon in their blood were divided into groups that received oral interferon or intraperitoneal interferon. In these experiments, mice that received oral interferon treatment were first trained during a 1-week training period to drink all of their daily water during a 1-hr period of time each day, according to the method of Segall and Crnic (17). Mice trained according to this method drink an average of 2.5 ml of water during the 1-hr period of time. Mice were given the oral interferon treatment in their drinking water together with 0.1% gelatin. At various times after the administration of the interferons, groups of mice were bled without the use of EDTA. The blood was allowed to clot and the cells were spun out of the serum using a microcentrifuge. The serum was titered for interferon activity as described above.

Results

Lack of Detection of Interferon in the Blood after Oral Administration of rHuIFN-αA/D. The relative abilities of intraperitoneally and orally administered interferons to become distributed in the blood were monitored. Mice were divided into two groups and treated either intraperitoneally or orally with 3×10^5 units of rHuIFN-αA/D. For mice treated intraperitoneally, the mice were inoculated with rHuIFN-αA/D in a volume of 0.2 ml. For mice treated orally, the mice were trained to consume all of their water in a 1-hr period. The interferon was delivered with the water and was consumed during the 1-hr period. The mice drank an average of 2.5 ml of interferon (1.2×10^5 units/ml). The mice were then bled at various times after the administration of the interferons and the serum was titered for the presence of interferon.

The averaged results of three experiments are shown in Figure 1A. It can be seen that, after intraperitoneal administration of rHuIFN-αA/D, approximately 5300 units/ml of interferon were detected in the blood within 30 min of injection. The interferon level in the blood declined thereafter. These results were similar to those observed by other investigators (18). In contrast to the results observed with intraperitoneal administration of rHuIFN-αA/D, interferon was not detected in the blood (<10 units/ml) after oral rHuIFN-αA/D administration.

Lack of Detection of Interferon in the Blood after Oral Administration of rMuIFN-γ. Mice were divided into two groups and treated either intraperitoneally or orally with 5×10^4 units of rMuIFN-γ, as described in the section above for rHuIFN-αA/D. Mice treated orally drank 2.5 ml of interferon (2×10^4 units/ml). The averaged results of two experiments are shown in Figure 1B. It can be seen that, after intraperitoneal administration of rMuIFN-γ, approximately 1600 units/ml of interferon were detected in the blood within 30 min. The level of interferon in the blood peaked at approximately 3200 units/ml at 60 min after injection and declined thereafter. These results were similar to those observed by other investigators (19). In contrast to the results observed with intraperitoneal administration of rMuIFN-γ, interferon was not detected in the blood (<5 units/ml) after oral rMuIFN-γ administration. The results with intraperitoneally and orally administered rMuIFN-γ were essentially the same as those observed with rHuIFN-αA/D.

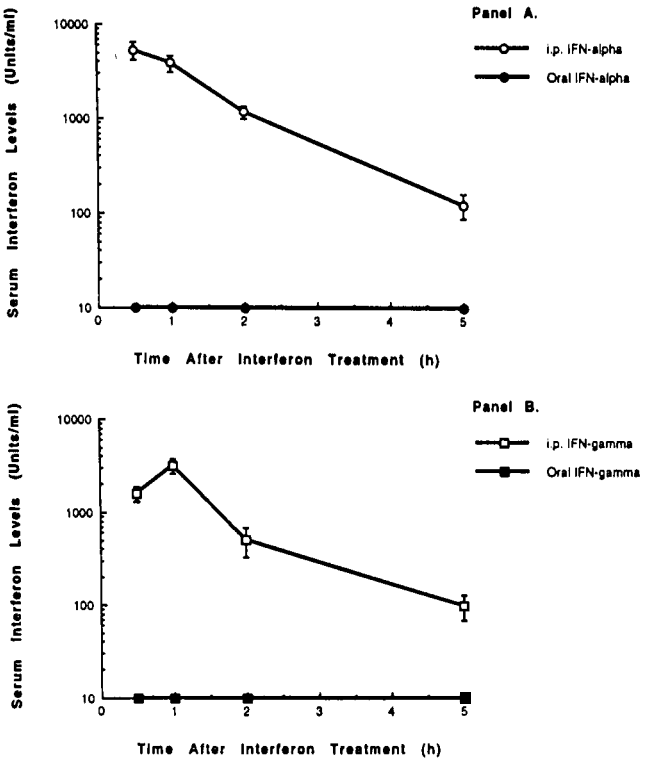

Figure 1. Serum levels of rHuIFN-αA/D and rMuIFN-γ after oral and intraperitoneal administration. Mice treated orally with rHuIFN-αA/D or rMuIFN-γ consumed the interferon in a 1-hr period. Mice treated intraperitoneally received the rHuIFN-αA/D or the rMuIFN-γ in a 0.2-ml injection. The mice were bled from the retro-orbital plexus at the indicated times after interferon treatment. The sera were collected and assayed for interferon activity. The serum interferon levels were plotted versus the time after interferon treatment. (A) Mice were treated either orally or intraperitoneally with 3×10^5 units of rHuIFN-αA/D. Each data point represents the mean ± SE of interferon titers from three experiments. (B) Mice were treated either orally or intraperitoneally with 5×10^4 units of rMuIFN-γ. Each data point represents the mean ± SE of interferon titers from two experiments.

Effect of Circulating Antibody to MuIFN-β on the Peripheral WBC Suppressive Effects of Intraperitoneally and Orally Administered MuIFN-β. The relative abilities of intraperitoneally and orally administered interferons to establish a suppression of the peripheral WBC count despite the presence in the blood of antibody to the interferon was monitored. The studies were performed initially with MuIFN-β rather than rHuIFN-αA/D because of the greater availability of antibody to MuIFN-β and because previous studies have shown that orally administered MuIFN-β and rHuIFN-αA/D give comparable levels of suppression of the peripheral WBC count (14). Mice were divided into two groups that were given a single intraperitoneal inoculation with either 1000 units of antibody to MuIFN-β or PBS. The two groups were then subdivided into three subgroups each. The six subgroups were treated for 3 days with intraperitoneally administered MuIFN-β (5000 units/day), orally administered MuIFN-β (5000 units/day), or both intraperitoneally with BSA/PBS and orally with gelatin/water (interferon carrier). The mice were then bled and their peripheral WBC counts were determined. The averaged results of two experiments are presented in Figure 2. As indicated in the Materials and Methods, only mice with >90 units/ml of circulating antibody to MuIFN-β were included in the results. It can be seen that the presence of circulating antibody to MuIFN-β did not affect the peripheral WBC counts of mice treated with interferon carrier (102% of the Day 0 count for mice treated with interferon carrier; 100% of the Day 0 count for mice treated with antibody to MuIFN-β and interferon carrier). Mice treated with PBS and intraperitoneal MuIFN-β developed a significant suppression of the peripheral WBC count (80% of the Day 0 count; $P < 0.0001$). Mice treated with antibody to MuIFN-β and intraperitoneal MuIFN-β did not develop a significant suppression of the peripheral WBC count (103% of the Day 0 count). Mice treated with PBS and oral MuIFN-β developed a significant suppression of the peripheral WBC count (86% of the Day 0 count; $P = 0.0006$). In contrast to the results with intraperitoneally administered MuIFN-β, mice treated with antibody to MuIFN-β and oral MuIFN-β developed a significant suppression of the peripheral WBC count (85% of the Day 0 count; $P = 0.0005$). This level of suppression was equivalent to the amount of suppression seen with PBS and oral MuIFN-β. Thus, the presence of circulating antibody to MuIFN-β blocked the peripheral WBC suppressive effect of intraperitoneally

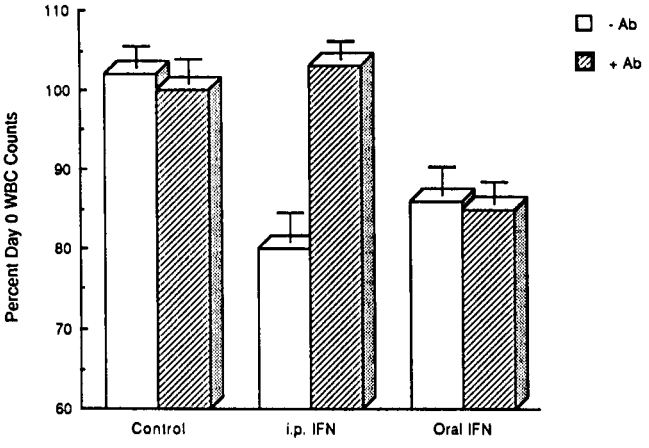

Figure 2. Effect of circulating antibody to MuIFN-β on MuIFN-β-induced peripheral WBC suppression after oral and intraperitoneal administration. Mice were inoculated with 1000 units of antibody to MuIFN-β or with PBS. Beginning 1 day later and continuing for 3 days, the mice were treated orally or intraperitoneally with 5000 units/day of MuIFN-β. Mice treated orally with MuIFN-β consumed the interferon in a 24-hr period. Mice treated intraperitoneally received the MuIFN-β in a 0.2-ml injection. WBC counts were made 1 day before antibody administration (Day 0) and after 3 days of MuIFN-β treatment (Day 4). The data were calculated as percentage of Day 0 values. The percentage of Day 0 WBC count was plotted versus the treatment given. Each bar represents the mean ± SE from two experiments.

administered, but not of orally administered, MuIFN-β.

Effect of Circulating Antibody to MuIFN-γ on the Peripheral WBC Suppressive Effects of Intraperitoneally and Orally Administered rMuIFN-γ. Mice were divided into two groups that were inoculated either intraperitoneally in one experiment or intravenously in a second experiment with a single injection of either 1000 units of antibody to MuIFN-γ or PBS, as described above. The two groups were then subdivided and treated for 3 days with intraperitoneally administered rMuIFN-γ (500 units/day), orally administered rMuIFN-γ (5000 units/day), or both intraperitoneally with BSA/PBS and orally with gelatin/water (interferon carrier). It can be noted that different concentrations have been used for intraperitoneally and orally treated mice, since it has been reported previously that intraperitoneally administered rMuIFN-γ was more potent than orally administered rMuIFN-γ (14). Thus, to achieve equal levels of suppression, mice were treated with 500 units/day of intraperitoneally administered rMuIFN-γ or with 5000 units/day of orally administered rMuIFN-γ. The mice were bled and their peripheral WBC counts were determined. No differences were noticed in the data obtained with intraperitoneal versus intravenous injection of antibody to MuIFN-γ. The averaged results of the two experiments are presented in Figure 3. As indicated in Materials and Methods, only mice with >60 units/ml of circulating antibody to MuIFN-γ were included in the results. It can be seen that the presence of circulating antibody to MuIFN-γ did not affect the peripheral WBC counts of mice treated with interferon carrier (110% of the Day 0 count for mice treated with interferon carrier; 102% of the Day 0 count for mice treated with antibody to MuIFN-γ and interferon carrier). Mice treated with PBS and intraperitoneal rMuIFN-γ developed a significant suppression of the peripheral WBC count (78% of the Day 0 count; $P < 0.0001$). Mice treated with antibody to MuIFN-γ and intraperitoneal rMuIFN-γ did not develop a significant suppression of the peripheral WBC count (100% of the Day 0 count). Mice treated with PBS and oral rMuIFN-γ developed a significant suppression of the peripheral WBC count (78% of the Day 0 count; $P < 0.0001$). In contrast to the results with intraperitoneally administered rMuIFN-γ, mice treated with antibody to MuIFN-γ and oral rMuIFN-γ developed a significant suppression of the peripheral WBC count (82% of the Day 0 count; $P < 0.0001$). Thus, the presence of circulating antibody to MuIFN-γ blocked the peripheral WBC suppressive effect of intraperitoneally administered, but not of orally administered, rMuIFN-γ. Furthermore, the results of these antibody studies with rMuIFN-γ paralleled those observed with MuIFN-β.

Transfer of the Peripheral WBC Suppressive Effects of Orally Administered Interferons by Peripheral Blood Cells. Since orally administered interferons could not be detected in the blood and since their WBC suppressive effects could not be blocked by circulating antibody, other mechanisms by which orally administered interferons exerted their systemic effects were evaluated. To address this point, the blood of donor mice that were mock treated or treated orally with interferons (5000 units/day of rHuIFN-αA/D or 5000 units/day of rMuIFN-γ) was evaluated for its ability to

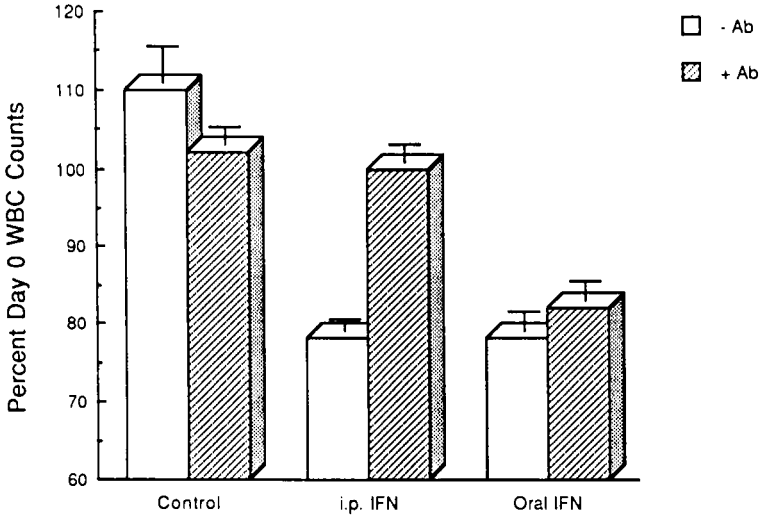

Figure 3. Effect of circulating antibody to MuIFN-γ on rMuIFN-γ-induced peripheral WBC suppression after oral and intraperitoneal administration. Mice were inoculated with 1000 units of antibody to MuIFN-γ or with PBS. Beginning 1 day later and continuing for 3 days, the mice were treated orally or intraperitoneally with rMuIFN-γ. Mice treated orally with 5000 units/day of rMuIFN-γ consumed the interferon in a 24-hr period. Mice treated intraperitoneally received 500 units of rMuIFN-γ in a 0.2-ml injection. WBC counts were made 1 day before antibody administration (Day 0) and after 3 days of MuIFN-γ treatment (Day 4). The data were calculated as percentage of Day 0 values. The percentage of Day 0 WBC count was plotted versus the treatment given. Each bar represents the mean ± SE from two experiments.

transfer the peripheral WBC suppressive effects. As described in Materials and Methods, mice were orally treated with interferon for 1 day, 2 days, and 3 days. The blood of the mice in these three groups was harvested on three successive days and divided by centrifugation into plasma and packed cells. Groups of recipient mice were treated for 3 successive days either with the plasma from the donor mice (equivalent to approximately 0.15 ml of undiluted plasma) or with the resuspended packed cells from the donor mice. It should be noted that each mouse received approximately 1×10^6 WBCs/day for 3 days. The recipient mice were then bled and their peripheral WBC counts determined. Table I presents the summarized data from two experiments. It can be seen that administration of plasma or cells from interferon carrier-treated donor mice had no suppressive effect on the peripheral WBC count of recipient mice. Also, administration of plasma from oral rHuIFN-αA/D- or rMuIFN-γ-treated donor mice had no suppressive effect on the peripheral WBC count of recipient mice. However, administration of cells from oral rHuIFN-αA/D- or rMuIFN-γ-treated donor mice significantly suppressed the peripheral WBC count of recipient mice. The results indicate that the WBC suppressive effects of both orally administered rHuIFN-αA/D and orally administered rMuIFN-γ are transferred from donor to recipient mice with the cell component of the blood.

Effect of Orally Administered rHuIFN-αA/D on the Various WBC Subpopulations. In view of the transfer of the WBC suppressive effects with the cell component of the blood, it was important to determine whether a particular subpopulation of WBC was most affected by interferon treatment. To address this question, mice were treated orally with rHuIFN-αA/D (5,000 units/day), intraperitoneally with rHuIFN-αA/D (5,000 units/day), or both orally with gelatin/water and intraperitoneally with BSA/PBS (interferon carrier) for 3 days. These interferon treatments caused significant suppression of the peripheral WBC count (79.7 ± 3.1% of Day 0 counts for intraperitoneally administered rHuIFN-αA/D and 88.2 ± 2.5% of Day 0 counts for orally administered rHuIFN-αA/D compared with 102.3 ± 4.3% of Day 0 counts for interferon carrier treatment [mean ± SE]). Differential counts were performed to enumerate the relative percentage of lymphocytes, neutrophils (polymorphonuclear leukocytes), and monocytes. Table II presents the results of a representative experiment. In agreement with previous observations (20), it can be seen that intraperitoneal administration of rHuIFN-αA/D did not cause a significant change in the relative percentage of each WBC subpopulation present in the blood. It can also be seen that oral administration of rHuIFN-αA/D did not cause a significant change in the relative percentage of each WBC subpopulation present in the blood. Thus, no specific subpopulation of WBC was suppressed to a greater extent than another.

Table I. Transfer of Peripheral WBC Suppressive Effect of rHuIFN-αA/D or rMuIFN-γ Administered Orally

Treatment	Percentage of day 0 control WBC (mean ± SE)
Donor mice treated with BSA in water	
Plasma from donor mice	104 ± 3.5
Packed cells from donor mice	104 ± 3.1
Donor mice treated with rHuIFN-αA/D (5000 units/day)	
Plasma from donor mice	101 ± 2.8 (NS)
Packed cells from donor mice	82 ± 2.0 ($P < 0.0001$)
Donor mice treated with rMuIFN-γ (5000 units/day)	
Plasma from donor mice	103 ± 3.8 (NS)
Packed cells from donor mice	79 ± 3.6 ($P < 0.0001$)

Discussion

Suppression of the peripheral WBC count has been recognized to be a side effect of interferon therapy in clinical trials (2) and in animal models (21, 22). These studies focused on the effects of subcutaneous or intravenously administered interferons. Recently, it was reported that orally administered interferons could also exert a suppressive effect on peripheral WBC counts in mice (14). Studies to determine the mechanism by which orally administered interferons exert their WBC suppressive effects in mice were initiated.

At least three mechanisms by which orally administered interferons exert their WBC suppressive effect in mice can be postulated. One mechanism could involve the direct absorption of interferon into the blood at the mucosal surfaces of the mouth or throat. This possibility was evaluated in two sets of experiments. In one set of experiments, interferon levels in the blood of mice were monitored after intraperitoneal and oral administration of rHuIFN-αA/D and rMuIFN-γ. Levels of rHuIFN-αA/D observed in the blood at 30 min and 60 min after intraperitoneal injection were 1.8% and 1.5% of the injected amount. These values correlate well with published values of 1.5% for rHuIFN-αA/D injected subcutaneously (18). The level of rMuIFN-γ observed in the blood at 60 min after intraperitoneal injection was 6.4%. This value compares very well with published values of 1.5% for rMuIFN-γ injected subcutaneously (19). Thus, the ability to detect interferons in the blood after intraperitoneal injection was comparable to that reported in the literature. However, it was not possible to detect either rHuIFN-αA/D or rMuIFN-γ in the serum after their oral administration.

Table II. Differential Counts in Mice Treated after Intraperitoneal and Oral Treatment with rHuIFN-α A/D

Treatment	White blood cell subpopulation (mean ± SE)		
	Lymphocytes	Neutrophils	Monocytes
Control	90.3 ± 1.3	7.8 ± 1.3	1.1 ± 0.5
IP rHuIFN-α A/D (5000 units/day)	89.6 ± 1.9 (NS)	8.3 ± 1.7 (NS)	1.7 ± 0.5 (NS)
Oral rHuIFN-α A/D (5000 units/day)	87.3 ± 1.5 (NS)	11.7 ± 1.6 (NS)	0.2 ± 0.1 (NS)

The observations that neither rHuIFN-αA/D nor rMuIFN-γ could be detected in the blood after their oral administration argue against this possible mechanism. Although unlikely, it was still possible that, after oral administration, undetectable levels of interferon were still circulating in the blood that were sufficiently potent to exert a peripheral WBC suppressive effect.

To address this possibility, another set of experiments were performed. Mice were pretreated with antibody to MuIFN-β or MuIFN-γ before the oral or intraperitoneal administration of MuIFN-β or rMuIFN-γ, respectively. Pretreatment with antibody to the interferons was observed to prevent the peripheral WBC suppressive effects of intraperitoneally administered interferons. Pretreatment with antibody to the interferons did not prevent the peripheral WBC suppressive effects of orally administered interferons. Thus, antibody to interferons blocked the peripheral WBC suppressive effects of intraperitoneally administered interferons that could be detected in significant levels in the blood. However, antibody did not block the peripheral WBC suppressive effects of orally administered interferons that could not be detected in the blood. These observations made it highly unlikely that, after oral administration, undetectable levels of interferon were still circulating in the blood and were able to exert a peripheral WBC suppressive effect. Taken together, the results of these two sets of experiments argue strongly against the direct absorption of interferon into the blood at the mucosal surfaces of the mouth or throat as a possible mechanism by which orally administered interferons could exert their peripheral WBC suppressive effects.

A second mechanism could involve the interferon-mediated activation of leukocytes in oropharyngeal mucosa or lymphoid tissue to produce lymphokines or cytokines that would then act to suppress the peripheral WBC count. To address this possibility, donor mice were orally treated with rHuIFN-αA/D or rMuIFN-γ and their plasma was harvested and administered to recipient mice. The administration of plasma from donor mice was found to have no peripheral WBC suppressive effect in recipient mice. Thus, it was unlikely that oral administration of interferons caused the production of lymphokines or cytokines which then acted through the blood to exert a peripheral WBC suppressive effect.

A third mechanism could involve the interferon-mediated activation of leukocytes in oropharyngeal mucosa or lymphoid tissue which would then pass through the blood to act directly to suppress the peripheral WBC count via a transfer mechanism, as suggested by Blalock and co-workers (23–26). To address this possibility, donor mice were orally treated with rHuIFN-αA/D or rMuIFN-γ and their packed blood cells were harvested and administered to recipient mice. The administration of blood cells from donor mice caused a significant peripheral WBC suppressive effect in recipient mice. Thus, it is most probable that oral administration of these interferons causes an interferon-mediated activation of leukocytes which then pass through the blood to exert a peripheral WBC suppressive effect.

The transfer effect is very potent. Peripheral WBC suppression was observed when each recipient mouse received approximately 10^6 donor mouse white blood cells. This represents only about 7% of the total peripheral WBC in a donor mouse. An examination of possible changes in the relative percentages of WBC subpopulations in the blood after oral interferon treatment might have given an indication of the specific subset of blood cell involved in mediating the transfer effect. However, oral interferon treatment did not show a preferential suppression of a particular WBC subpopulation. Thus, the identity of the specific subset of cells responsible for transferring the peripheral WBC suppressive activity is unknown at this time and is the subject of continuing study.

Whatever the subset of cells, the observation that peripheral WBC suppression induced by orally administered interferons could be transferred with blood cells from donor mice provides definitive evidence that the peripheral WBC suppression induced by orally administered interferons is a systemic effect. This observation also raises the possibility that orally administered interferons may exert other systemic effects. Efforts are underway to determine whether other systemic effects of orally administered interferons, such as antitumor activity, can be demonstrated.

It is clear that intraperitoneally and orally administered interferons exert their WBC suppressive effects through different mechanisms: one mediated by free interferon in the blood and the other mediated by "activated" leukocytes. The question remains whether orally administered interferons suppress the peripheral

WBC count by destroying circulating WBC, by increasing the trafficking of WBC out of the circulation, or by suppressing the WBC-producing function of the bone marrow. Unpublished evidence suggests that the ultimate effect of orally administered interferons is the same as that of intraperitoneally administered interferons. Interferons administered by both routes of administration appear to achieve their WBC suppressive effects through a suppression of the bone marrow (Koren and Fleischmann, unpublished observations).

This work was supported by a grant from Amarillo Cell Culture Co., Inc.; by U.S. Public Health Service Grant No. CA50752 awarded by the National Cancer Institute, Department of Health and Human Services; by The John Sealy Memorial Endowment Fund; and by a James W. McLaughlin Fellowship (C. M. F.).

1. Baron S, Tyring SK, Fleischmann WR Jr, Coppenhaver DH, Niesel DW, Klimpel GR, Stanton GJ, Hughes TK. Interferon: Clinical use, mechanisms and future potential. JAMA **266**:1375–1383, 1991.
2. Billiau A. Interferon therapy: Pharmacokinetic and pharmacological aspects. Arch Virol **67**:121–133, 1981.
3. Schafer TW, Lieberman M, Cohen M, Came PE. Interferon administered orally: Protection of neonatal mice from lethal virus challenge. Science **176**:1326–1327, 1972.
4. Merigan TC, Hall TS, Reed SE, Tyrell DAJ. Inhibition of respiratory virus infection by locally applied interferon. Lancet **1**:563–567, 1973.
5. Greenberg SB, Harmon MW, Johnson PE, Couch RB. Antiviral activity of intranasally applied human leukocyte interferon. Antimicrob Agents Chemother **14**:596–600, 1978.
6. Cummins JM, Rosenquist BD. Protection of calves against rhinovirus infection by nasal secretion interferon induced by infectious bovine rhinotracheitis virus. Am J Vet Res **41**:161–165, 1980.
7. Higgins PG, Phillpotts RJ, Scott GM, Wallace J, Bernhardt LL, Tyrell DAJ. Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrob Agents Chemother **24**:713–715, 1983.
8. Turner RB, Felton A, Kosak K, Kelsey DK, Meschievitz CK. Prevention of experimental coronavirus colds with intranasal alpha-2b interferon. J Infect Dis **154**:443–447, 1986.
9. Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM. Prevention of natural colds by contact prophylaxis with intranasal alpha2-interferon. N Engl J Med **314**:71–75, 1986.
10. Smith AL, Barthold SW, Beck DS. Intranasally administered alpha/beta interferon prevents extension of mouse hepatitis virus, strain JHM, into the brains of BALB/cByJ mice. Antiviral Res **8**:239–246, 1987.
11. Cummins JM, Tompkins MB, Olsen RG, Tompkins WA, Lewis MG. Oral use of human alpha interferon in cats. J Biol Response Mod **7**:513–523, 1988.
12. Hutchinson V, Cummins JM. Low-dose oral interferon in patient with AIDS. Lancet **2**:1530–1531, 1987.
13. Koech DK, Obel AO, Minowada J, Hutchinson VA, Cummins JM. Low dose oral alpha-interferon therapy for patients seropositive for the human immunodeficiency virus type-1 (HIV-1). Mol Biother **2**:91–95, 1990.
14. Fleischmann WR Jr, Fields EE, Wang J-L, Hughes TK, Stanton GJ. Modulation of peripheral leukocyte counts in mice by oral administration of interferons. Proc Soc Exp Biol Med **197**:424–430, 1991.
15. Campbell JB, Grunberger T, Kochman MA, White SL. A microplaque reduction assay for human and mouse interferon. Can J Microbiol **21**:1247–1253, 1975.
16. Havell EA, Spitalny GL. Production and classification of anti-murine interferon-gamma sera. J Interferon Res **3**:191–198, 1983.
17. Segall MA, Crnic LS. A test of conditioned taste aversion with mouse IFN-α. Brain Behav Immun **4**:223–231, 1990.
18. Ramani P, Balkwill FR. Action of recombinant alpha interferon against experimental and spontaneous metastases in a murine model. Int J Cancer **43**:140–146, 1989.
19. Maekawa R, Kitagawa T, Hojo K, Wada T, Sato K. Distinct antitumor mechanisms of recombinant murine interferon-γ against two murine tumor models. J Interferon Res **8**:227–239, 1988.
20. Koren S, Klimpel GR, Fleischmann WR Jr. Treatment of mice with macrophage colony stimulating factor (CSF-1) prevents the in vivo myelosuppression induced by murine alpha, beta, and gamma interferons. J Biol Response Mod **5**:481–489, 1986.
21. Degre M. Influence of exogenous interferon on the peripheral white blood cell count in mice. Int J Cancer **14**:699–703, 1974.
22. Gresser I, Guy-Grand D, Maury C, Maunoury M-T. Interferon induces peripheral lymphadenopathy in mice. J Immunol **127**:1569–1575, 1981.
23. Blalock JE, Baron S. Interferon-induced transfer of viral resistance between animal cells. Nature **269**:422–425, 1980.
24. Blalock JE. A small fraction of cells communicates the maximal interferon sensitivity to a population. Proc Soc Exp Biol Med **162**:80–84, 1979.
25. Blalock JE, Weigent DA, Langford MP, Stanton GJ. Transfer of interferon-induced viral resistance from human leukocytes to other cell types. Infect Immun **29**:356–360, 1980.
26. Lloyd RE, Blalock JE, Stanton GJ. Cell to cell transfer of interferon-inducing antiproliferative activity. Science **221**:953–955, 1983.