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Murine retroviral disease-enhancing effects of a pyrimidinone immunomodulator

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Summary

(B10.A × A/WySn)F_1 mice, infected with the Friend virus (FV) complex, were used as a predictive therapeutic model for AIDS. These infected mice exhibit many of the viral and immunologic manifestations of AIDS. Bropirimine (2-amino-5-bromo-6-phenyl-4[^{3}H]pyrimidinone, ABPP) is an immunomodulating compound which has been shown to inhibit other viral infections. Oral (per os treatment) dosages of ABPP ranging from 50 to 400 mg/kg/day for 3 days resulted in increased numbers of infectious centers in the infected mice and increased splenomegaly and percentage of Ig+ (B cells) in spleens of infected and uninfected mice. Decreased percentages of total Thy-1.2+ (total T) cells and L3T4+ (T-helper) cells were seen in both uninfected and infected mice and a slightly decreased percentage of Ly-2+ (T-suppressor/cytotoxic) cells was observed in spleens of the infected mice. No effect on Ly2+ cells in spleens of uninfected mice was found. Intraperitoneal injection, single or multiple, of 20–200 mg/kg ABPP prior to FV injection resulted in increased spleen weights but had no effect on numbers of infectious centers in the spleens or on FV antibody titers in the plasma. Intraperitoneal treatment of uninfected mice with ABPP resulted in slight or no changes in percentages of Thy-1.2+, L3T4+ and Ly-2+ cells. Mice receiving multiple exposures of ABPP had an increase in percentage of splenic B cells and a depressed response to the T cell mitogen PHA. Treatment with ABPP induced the production of interferon (IFN); however, a state of hyporesponsive IFN production was seen following multiple administrations of ABPP. These data suggest that the immunomodulator ABPP may have an enhancing effect on this retroviral disease.

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AIDS; HIV; Friend virus; Biological response modifier; Bropirimine; Pyrimidinone

Introduction

Many substances, including immune modulators, are being investigated for their potential therapeutic efficacy for human immunodeficiency virus (HIV) infection. One of these compounds, bropirimine (2-amino-5-bromo-6-phenyl-4[^{3}H]pyrimidinone, ABPP) is a synthetic immunomodulator which has been reported to stimulate interferon production (Stringfellow et al., 1980), natural killer cell activity (Lotzova et al., 1983), macrophage activity (Wierenga, 1985) and antibody production (Fast et al., 1982). Moreover, this material has been shown to have in vivo antiviral activities against infections induced by such viruses as herpes simplex types 1 and 2, murine cytomegalo parainfluenza type 3 and infectious bovine rhinotracheitis (Wierenga, 1985). We have previously shown ABPP to also significantly inhibit Punta Toro virus infections in mice (Sidwell et al., 1990).

This study investigated the potential antiviral and immunomodulatory activities of ABPP in a retroviral infection model which has been developed as a therapeutic model for HIV infection (Morrey et al., 1991). In this model, Friend virus (FV) infection of mice of one particular strain, (B10.A × A/WySn)F_1, having a genetic constitution of Rfv-3r/s, H-2a/a, FV-3r/s, shares several of the characteristics of HIV infection in man. In both infections, immunosuppression of the adult host occurs, anti-retroviral antibodies are present despite an immunologically compromised state and only low levels of infectious virus are seen once the antibodies develop (Chesebro and Wehrly, 1979). Moreover, FV-infected mice exhibit reduced percentages of splenic T (Thy 1.2+) cells, helper T (L3T4+) cells and suppressor/cytotoxic T (Lyt 2+) cells and depressed response to phytohemagglutinin (PHA) (Morrey et al., 1991).

Materials and Methods

Virus

The Lilly-Steeves B-tropic strain of FV complex (obtained from Dr. Bruce Chesebro, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana) which consists of helper Friend murine leukemia virus (F-MuLV) and defective spleen focus-forming virus (SFFV) was used. A cell-free pool of this complex was prepared from spleen homogenates of infected mice, stored at −70°C and titrated for infectivity in mice.

Mice

Young adult (B10.A × A/WySn)F_1 mice, 19–22 g, were obtained from

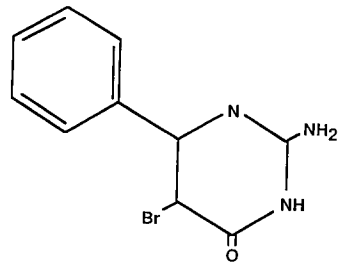

Fig. 1. 2-Amino-5-bromo-6-phenyl-4[^{3}H]pyrimidinone (ABPP, bropirimine).

Simonsen Laboratories (Gilroy, CA). These animals were produced by mating female B10.A with male A/WySn mice (Jackson Laboratories, Bar Harbor, ME). The genetic constitution of these mice was confirmed by Dr. H.A. Hoffman (Animal Genetic Systems Co., Rockville, MD).

Bropirimine

The ABPP used (Fig. 1) was provided by Dr. Harold Renis, The UpJohn Co., Kalamazoo, MI. After storage at room temperature, this compound was diluted in 0.4% carboxymethylcellulose (CMC) to achieve the concentrations used in these experiments.

Viral parameters

Spleen weight, viral infectious centers in the spleen and cell-free virus in the plasma were indicators of virus infection. Infectious centers in the spleen and cell-free virus in the plasma were quantitated with a focal immunofluorescent assay (FIA) as described previously (Morrey et al., 1991). This assay employed a monoclonal antibody (provided by Dr. Bruce Chesebro), designated as MAb 48, which is specific to the envelope protein of F-MuLV. Spleen cells were co-cultivated as infectious centers with *Mus dunni* cells for 24 h. The *M. dunni* cells were grown to confluency over a 5-day period and incubated with the monoclonal antibody, rinsed and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Sigma Chemical Co., St. Louis, MO) to identify the focus forming units. To assay FV in plasma and cell-free supernatants of the spleen, serial dilutions of heparinized plasma or supernatant were incubated for 18 h with the *M. dunni* cells and the fluorescent foci in the cells were determined as above.

Cell preparation and enumeration

Spleens suspended in RPMI-1640 (GIBCO Laboratories, Grand Island, NY) were disassociated with a tissue homogenizer. Some of the spleen cell preparations (used in Expt. 1, described below) were pipetted into nylon wool columns and incubated for 1 h at 37°C. Non-adherent cells were eluted from the columns with warm RPMI-1640. Splenic lymphocytes were incubated at room temperature for 30 min with the monoclonal antibodies anti-Thy 1.2

(T-cells), anti-L3T4 (helper T-cells), anti-Lyt 2 (suppressor/cytotoxic T-cells), and goat anti-mouse Ig (B-cells). The percentage of labeled cells was determined with an EPICS-C flow cytometer (Coulter Electronics Inc., Hialeah, FL) equipped with an argon laser tuned to 400 mW at the 488 nm line. Cells were carried in Isoflow sheath fluid (Coulter) through a 76-μm flow tip and fluorescent signals were obtained using a bit-map format, gated on the forward light scatter versus right-angle scatter histogram. Fluorescent signals were processed through a three-decade log amplifier and displayed on a 256-channel scale. Fluorescence intensity was standardized using 10 μm latex beads (Immunocheck, Coulter) and adjusting the laser power to place the log-green histogram in channel 119.

T-cell blastogenesis assay

Spleen cells (2×10^5) were pipetted in triplicate into wells of flat-bottom 96-well microplates (Corning, Corning, NY) in a volume of 0.1 ml. Phytohemagglutinin (PHA, GIBCO) of various concentrations in medium containing 20% FBS were added to each well in 0.1-ml aliquots. The plates were incubated 48 h at 37°C. During the last 24 h of incubation, the cells were pulsed with 0.4 μCi of [^{3}H]thymidine. The cells were then harvested on glass fiber filter paper disks using a Skatron cell harvester (Flow Labs, Irvine, CA) and the uptake of radioactivity determined using a Packard Tri-Carb 1500 liquid scintillation counter. Since murine T-cells were normally relatively weak responders, maximizing of total incorporation was achieved by optimizing the length of incubation with PHA, the number of responding T-cells and the length of exposure to [^{3}H]thymidine.

Interferon assay

Serum samples to be assayed for interferon (IFN) were subjected to a series of dilutions in test medium (minimum essential medium with 2% fetal bovine serum, 0.18% $NaHCO_3$ and 50 μg/ml gentamicin) and 0.1 ml placed on an 18-h monolayer of mouse L cells in 96-well microplates and allowed to incubate for 24 h at 37°C. The cells were then drained and 0.1 ml of a 10^3 50% cell culture infectious doses of vesicular stomatitis virus (strain Indiana) was added to each. The plates were then incubated at 37°C for up to 6 days. Viral cytopathic effect (CPE) was read microscopically on days 3 to 6. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited the viral CPE by 50% or greater. A standard IFN sample with known titer was run in parallel as control, with the CPE readings determined when this sample achieved the expected titer. Virus controls, which were cells exposed to test medium and then to virus, and cell controls which were exposed to test medium only, were run in parallel.

Design of experiments

Three experiments were performed in order to determine the effects of ABPP in mice infected with 3.8×10^4 50% infectious doses (IDSO) of FV. Expt. 1:

ABPP in dosages of 400, 200, 100 and 50 mg/kg/day was administered by oral gavage (p.o.) daily for 3 days beginning 4 h after virus injection. Expt. 2: ABPP in the same concentrations as above was injected intraperitoneally (i.p.) once every 4 days for 12 days beginning 1 day prior to FV injection. Expt. 3: ABPP in dosages of 200, 64 and 30 mg/kg/day was administered i.p. in a single injection 4 h pre-virus inoculation. In all experiments, 4 groups of mice were studied: (a) FV-infected, ABPP-treated; (b) sham-infected, ABPP-treated (toxicity controls); (c) FV-infected, placebo-treated (virus controls); and (d) uninfected, untreated mice (normal controls). The toxicity and normal controls were weighed immediately before the first treatment and 18 h after final treatment. This early time point (day 4) for Expt. 1 was selected to measure immediate immunologic and virologic effects on the animals; the 24-h post-treatment examination time was consistent with all treatment regimens to allow comparisons between regimens. One-half of the mice in Expt. 1 and all animals in Expt. 2 were sacrificed 24 h after final treatment and their blood and spleen samples assayed for viral and immunologic parameters. The remainder of the mice in Expt. 1 were assayed at day 24 for viral and immunologic parameters. This latter assay time was selected to determine treatment effects at the time considered to be when peak plasma virus titers would be achieved (Morrey et al., 1990). The animals in Expt. 3 were assayed 12 days after viral inoculation to determine effects on viral parameters. This day of assay was selected to correlate with the study of Expt. 2. Differences in mean values (spleen weights, infectious centers in spleen, FV in plasma and cell enumeration) were analyzed by Student's *t*-test.

Results

Effects of oral administration of ABPP on FV disease

All dosages of ABPP, when given daily for 3 days, appeared to enhance the FV infection at 4 days after virus injection (Table 1). The infected mice receiving the highest dosage (400 mg/kg/day) of ABPP had a mean infectious center titer which was 2 $\log_{10}$ greater than that of the sham-treated, infected mice. Mean spleen weights of the infected mice were also significantly increased following treatment with all of the dosages of ABPP. FV in the plasma and in cell-free supernatants of the spleen were not detectable at this early time period after infection. Toxicity control mice receiving ABPP for 3 days also had significantly ($P < 0.01$) increased mean spleen weights on day 4 at the 400 and 200 mg/kg/day dosages (Table 1). The ABPP therapy had no effect on survival of the infected and uninfected hosts and did not alter weight changes in the toxicity controls at the dosages used. Plasma virus was not detectable at this early sampling time.

The disease-enhancing effects of ABPP seen 4 days after virus inoculation were less evident on day 24 (Table 1). At this time, splenomegaly enhancement was seen only at the 100 mg/kg/day ABPP dosage level. No decrease in

TABLE 1

Effect of Multiple Oral ABPP Treatment[a] on Viral Parameters Assayed 4 or 24 Days After FV Inoculation

Treatment	Dosage (mg/kg/day)	Toxicity controls		Infected, treated		
		Host wt. change (g)[b]	Mean spleen wt.[c] (mg ± S.E.)	Mean spleen wt. (mg ± S.E.)	Mean inf. ctrs.in spleen[d] (log_{10} ± S.E.)	Mean plasma[e] virus (log_{10} ± S.E.)
Viral assays performed Day 4 post-virus inoculation						
ABPP	400	0.7	117 ± 7**	118 ± 8**	4.6 ± 0.1**	<1.0
	200	1.1	110 ± 6**	105 ± 10*	3.3 ± 0.3**	<1.0
	100	0.8	65 ± 2	111 ± 3**	3.2 ± 0.3*	<1.0
	50	1.2	70 ± 7	111 ± 3**	3.3 ± 0.1**	<1.0
CMC	–	–	–	80 ± 9	2.5 ± 0.2	<1.0
Normals	–	1.0	85 ± 5	–	–	–
Viral assays performed Day 24 post-virus inoculation						
ABPP	400	0.7	117 ± 7**	1003 ± 205	2.7 ± 0.3	2.2 ± 0.3
	200	1.1	110 ± 6**	1066 ± 184	3.4 ± 0.3*	2.3 ± 0.3
	100	0.8	65 ± 2	1446 ± 123*	3.3 ± 0.2*	2.4 ± 0.2
	50	1.2	70 ± 7	964 ± 280	2.9 ± 0.3	1.9 ± 0.3
CMC	–	–	–	1030 ± 198	2.7 ± 0.3	2.1 ± 0.2
Normals	–	1.0	85 ± 5	–	–	–

[a]Once daily for 3 days beginning 4 h after virus inoculation.
[b]Difference between initial weight and weight 24 h following treatment termination.
[c]Arithmetic mean ± 2 standard errors determined 24 h following treatment termination. $n=4$ for toxicity controls; 10 for infected, treated; 16 for CMC-treated, infected, day 24 assay; 6 for normals.
[d]Geometric mean infectious cells/10^6 spleen cells ± 2 standard errors.
[e]Geometric mean focus-forming units/ml heparinized plasma ± 2 standard errors.
*P <0.05; **P <0.01.

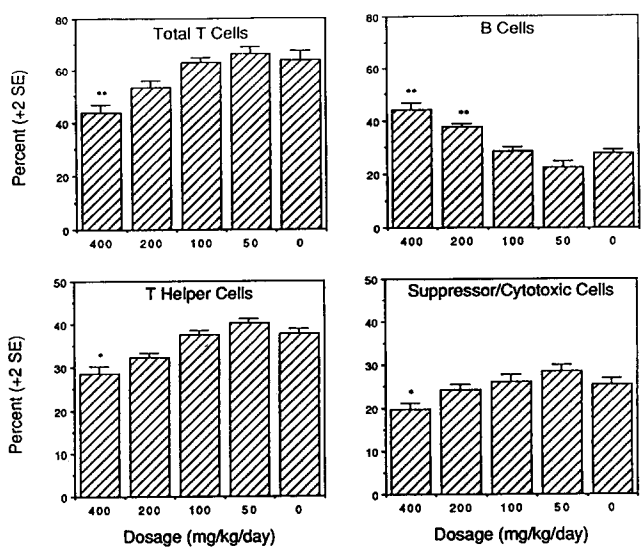

Fig. 2. Effects of oral ABPP treatment on cell populations of spleens of mice assayed 4 days after FV infection. *P<0.05 and **P<0.01 as compared to placebo-treated controls, $n=10$ for ABPP-treated animals, 20 for placebo-treated controls.

TABLE 2

Effect of Multiple i.p. ABPP Treatments[a] on viral parameters in FV-infected mice

Treatment	Dosage (mg/kg/day)	Toxicity controls		Infected, treated		
		Host wt. change (g)[c]	Mean spleen wt.[d] (mg ± 2 S.E.)	Mean spleen wt. (mg ± 2 S.E.)	Mean inf. ctrs.in spleen[e] (log_{10} ± 2 S.E.	Mean plasma[f] virus (log_{10} ± 2 S.E.)
ABPP	400	2.3	225 ± 21.6**	1123 ± 550	3.2 ± 0.3	2.8 ± 0.5
	200	2.0	175 ± 8.0**	1045 ± 280	3.1 ± 0.4	2.8 ± 0.6
	100	2.3	151 ± 14.1**	1074 ± 371	3.2 ± 0.3	2.9 ± 0.6
	50	1.8	146 ± 10.4**	1448 ± 312*	3.2 ± 0.4	2.7 ± 0.7
CMC	–	–	–	1083 ± 167	3.0 ± 0.2	3.2 ± 0.6
Normals	–	0.1	102 ± 8.3	–	–	–

[a]Injections every 4th day through day 11 beginning 24 h pre-virus inoculation.
[b]Animals sacrificed on day 12, 24 h after final treatment.
[c]Difference between initial weight and weight 24 h following treatment termination.
[d]Arithmetic mean ± 2 standard errors determined 24 h following treatment termination. $n = 10$ for ABPP-treated mice; 20 for CMC-treated; 4 for normals.
[e]Geometric mean infectious cells/10^6 spleen cells ± 2 standard errors.
[f]Geometric mean focus-forming units/ml heparinized plasma ± 2 standard errors.
*$P < 0.05$; **$P < 0.01$.

infectious centers in the spleen were seen, and, indeed, increases were observed in mice treated with 200 and 100 mg/kg/day dosages. Moderate but statistically insignificant plasma virus titers were also seen in the ABPP-treated animals.

Infected mice receiving 400 mg/kg/day of ABPP p.o. displayed a significantly decreased percentage of total T-cells as well as decreased percentages of helper T-cells and suppressor/cytotoxic T-cells (Fig. 2). In contrast, an increased percentage of B-cells was found in mice receiving 400 and 200 mg/kg/day. A similar pattern of changes in T- and B-cell percentages was seen in uninfected

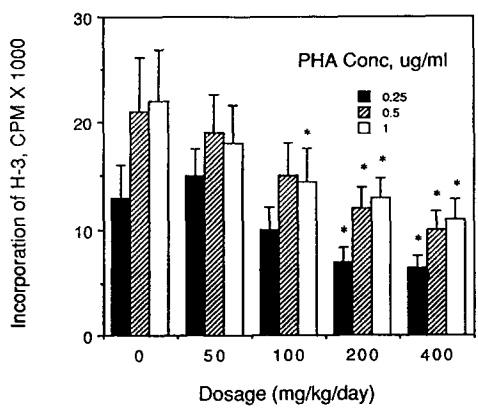

Fig. 3. Effects of i.p. ABPP treatment on PHA-induced response (incorporation of tritiated-thymidine) in spleen cells of uninfected mice treated on days 0, 4, 8 and 12. $P < 0.05$ as compared to placebo-treated controls.

TABLE 3

Effect of a single i.p. ABPP treatment[a] on viral parameters in FV-infected mice

Treatment	Dosage (mg/kg/day)	Toxicity controls	Infected, treated		
		Mean spleen wt.[b] (mg ± 2 S.E.)	Mean spleen wt. (mg ± 2 S.E.)	Mean inf. ctrs. in spleen[c] (log_{10} ± 2 S.E.)	Mean plasma[d] virus (log_{10} ± S.E.)
ABPP	200	86 ± 13.7	931 ± 283.0*	3.9 ± 0.3	5.0 ± 0.7
	64	96 ± 8.5	403 ± 63.1	4.1 ± 0.3	4.0 ± 0.3*
	30	95 ± 9.6	412 ± 113.6	3.6 ± 0.3	4.3 ± 0.6
CMC	–	–	497 ± 107.6	3.6 ± 0.4	4.7 ± 0.4
Normals	–	84 ± 9.2	–	–	–

[a]One injection 4 h pre-virus inoculation.
[b]Arithmetic mean ± 2 standard errors determined 24 h following treatment termination. $n = 10$ for ABPP-treated mice; 20 for CMC-treated; 4 for normals.
[c]Geometric mean infectious cells/10^6 spleen cells ± 2 standard errors.
[d]Geometric mean focus-forming units/ml heparinized plasma ± 2 standard errors.
*$P < 0.05$.

mice receiving the higher dosages of ABPP, except that the percentage of suppressor/cytotoxic T-cells was not decreased in these mice (data not presented).

At day 24 following virus inoculation, the ABPP treatment which terminated 20 days earlier did not have a measurable effect on the FV-induced decreases in percentages of total T-cells, helper T-cells and suppressor T-cells, nor the increase in percentage of B cells (data not presented).

Effects of intraperitoneal administration of ABPP on FV disease

The results of i.p. ABPP treatment administered every 4 days for 11 days (Expt. 2) are given in Table 2. The highest and lowest dosages caused slightly increased spleen weights in infected mice; a dose-dependent increase in spleen weights in uninfected mice was also observed. Although not statistically significant, a moderate increase in splenic infectious center titers was seen in infected, ABPP-treated mice. The ABPP doses used were not toxic as seen by no deaths or altered weight changes in the toxicity controls. ABPP had a dose-dependent suppressive effect on the response of T-cells to PHA (Fig. 3), but did not significantly decrease the T-cell populations in this experiment (data not presented).

The effects of ABPP on viral parameters on day 12 following a single i.p. ABPP treatment 4 h before FV injection (Expt. 3) are summarized in Table 3. Spleen weights were significantly increased in infected mice treated with 200 mg/kg, the highest dosage, of ABPP. Infectious centers in the spleen were moderately increased with the two highest dosages of ABPP; FV titers in the plasma were increased at the highest dose but reduced in mice receiving 64 mg/kg. This latter observation, being out of harmony with the rest of the data, may be spurious. The spleen weights of uninfected mice receiving ABPP were not appreciably changed.

TABLE 4

Murine interferon induction by ABPP

Treatment	Dosage (mg/kg)	Total treatments	Mean IFN titer[a] (log_{10} units/0.1 ml)
Treatment once daily for 3 days			
ABPP	400	1	3.3
	50	1	2.5
	400	2	2.8
	50	2	1.9
	400	3	0.2
	50	3	1.1
Treatment every 4 days for 3 treatments			
ABPP	400	1	3.5
	50	1	2.2
	400	2	3.1
	50	2	0.9
	400	3	2.9
	50	3	<1.0
Normals	–	–	<1.0

[a]IFN assay 90 min after treatment.

Interferon induction by ABPP

Two experiments were run to determine the ability of i.p. administered ABPP to induce IFN in uninfected mice using the once daily and the every 3 day treatment regimens. Two dosages of ABPP, 400 and 50 mg/kg/day, were used. Five mice were killed and their serum assayed for IFN 90 min after the last treatment. As seen in Table 4, strong IFN stimulation was seen after 1 and 2 daily ABPP treatments. After the third treatment, however, the IFN induction was markedly reduced, especially in the mice receiving the high ABPP dosage. Spacing the ABPP treatments 3 days apart allowed the high dosage of ABPP to induce high IFN levels through 3 separate treatments; the mice receiving the 50 mg/kg/day dose, however, produced significantly less IFN after the later therapies, with none detectable after the third treatment.

Discussion

The Friend virus complex has received extensive biological and molecular characterization over the years (Chesebro and Wehrly, 1979; Morrison et al., 1986; Morrison et al., 1987) particularly regarding the genes that regulate susceptibility to FV infection (Morrison et al., 1986). Alleles at the Rfv-3, H-2 and FV-3 loci appear to control production of anti-FV antibody coupled with recovery from viremia, recovery from FV-induced erythroleukemic splenomegaly, and extent of FV-induced immunosuppression (Chesebro et al., 1979), respectively. In a previous report we have extended these observations to also include a number of immunologic parameters (Morrey et al., 1990) so that this

murine retroviral model can be used to initially study the anti-HIV disease potential of immunomodulators.

In the current study, we found that ABPP, despite its reported widespread immunostimulatory (Tracey and Richard, 1986; Tracey et al., 1985; Richard et al., 1987) and anti-viral activities (Stringfellow, 1981; Skulnick et al., 1985; Sidwell et al., 1990), did not reduce viral or disease parameters in the retroviral model. Conversely, some dosages of ABPP, when administered p.o. or i.p., enhanced viral infection at certain sampling times. Furthermore, ABPP was not able to reverse the alterations in T- and B-cell profiles induced by FV-infection; in some cases treatment with ABPP appeared to cause further reduction in T-cell percentages and an increase in B-cell percentage. Finally, i.p. administration of ABPP had a suppressive effect on the response of lymphocytes to PHA, suggesting that ABPP may interfere with T-cell function. However, the reduced responsiveness to PHA may simply reflect the decrease in T-cell numbers (seen in the cell enumeration studies) rather than an actual defect in T-cell response. The 3 treatment regimens used in this study were selected based on our observations in another virus model where ABPP rendered significant antiviral effects using these or similar regimens (Sidwell et al., 1990).

An earlier study explored the effect of pyran, a synthetic polyanionic immunomodulator on mice infected with FV. Interperitoneal administration of pyran resulted in a protection against virus challenge. In contrast, i.v. treatment with pyran significantly enhanced splenomegaly and slightly decreased survival time (Schuller et al., 1975). This earlier study and our current findings illustrate the need to elucidate the mechanisms of action of immunomodulators before using them in therapy.

The IFN induction by i.p. ABPP injection seen in this study confirms earlier reports (Wierenga, 1985; Nichol et al., 1976) of the rapid and potent IFN induction by this compound. Nichol et al. (1976) have reported p.o., i.p. and subcutaneous ABPP administration to yield nearly equivalent IFN induction in mice. It was anticipated that a hyporesponsive IFN productive state may develop following multiple administrations with ABPP; this was clearly seen in the present experiment when the drug was administered daily, although IFN kinetics data may have illustrated this effect more clearly if they had been run. Spacing the treatments 3 days apart reduced this hyporesponse, although it was apparently still evident using the 50 mg/kg/day dose of ABPP.

The mechanism by which ABPP treatment caused an apparent enhancement of the FV infection in this study is not clear. It is known that exogenous IFN has a suppressive effect on murine retroviral infections (Ruprecht et al., 1990; Sidwell et al., 1991); that disease suppression was not seen in the present experiments using IFN inducers. Indeed, the amplified splenomegaly observed in the present study following ABPP treatment has also been seen using poly I:C, another recognized IFN inducer. Larson et al. (1969) has reported that the latter material, when administered to mice prior to FV inoculation, caused a dose-related splenomegaly. Perhaps exposure to IFN induction at an inappropriate time upsets the normal immunoregulatory mechanisms of the

host, resulting in a reduced specific response to FV, but direct evidence for this is lacking. It was thought the hyporesponsiveness in IFN production following multiple ABPP therapy may be a factor in this disease enhancement. However, the single ABPP treatment, which is not associated with hyporesponsiveness, also resulted in a moderate disease enhancement. No direct evidence is available from the present studies to show an actual relationship between IFN induction and FV disease enhancement. Another possibility is that ABPP enhances FV infection in a manner similar to that in which the expression of HIV in infected T-cell lines can be induced with a broad range of activation signals, including mitogens (Clouse et al., 1989), antigen (Clouse et al., 1989), phorbol esters (Clouse et al., 1989) and tumor necrosis factor (Israel et al., 1989).

An immunologic effect of ABPP seen in this study which should be focused upon in considering the apparent enhancement of FV disease is the increased splenic B-cells resulting from ABPP treatment. HIV infection is known to cause specific B-cell activation, resulting in high numbers of immunoglobulin-secreting cells in the circulation (Arradori et al., 1989). This specific antibody may have pathogenic consequences by contributing to immune damage through antibody-dependent cell-mediated cytotoxicity of HIV-infected T-cells (Rook et al., 1987) and/or to the generation of specific suppressor cells. The increased numbers of B-cells may also serve as additional target cells for the virus, as we have reviewed in another publication (Morrey et al., 1991) in which the immunomodulator imexon, which reduced B cell population in FV-infected mice, also was significantly inhibitory to the virus disease.

Work is continuing to develop the potential clinical uses of ABPP. The drug has recently been approved for clinical use in Japan against certain forms of human cancer, the anti-cancer effects primarily attributed to the drug's IFN-inducing properties (personal communication, Dr. Harold Renis, The UpJohn Co., Kalamazoo, MI). The findings of the present study suggest caution in the use of such compounds in treating retroviral infections, however.

Acknowledgements

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