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Effect of imexon treatment on Friend virus complex infection using genetically defined mice as a model for HIV-1 infection

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Summary

Imexon (4-imino-1,4-diazobicyclo-3.1.0-hexan-2-one) was moderately effective in the treatment of a retroviral infection in a genetically defined murine model. The animal model consisted of a Friend virus complex (FV) infection in a hybrid mouse strain, (B10.A × A/WySn)F_1, which has similarities with acquired immune deficiency syndrome (AIDS). Intraperitoneal imexon initiated 1 or 3 days after FV inoculation and continued through 13 days after inoculation significantly reduced splenomegaly, splenic cell-free virus titers and viral RNA. Viral infectious centers/10^6 splenocytes and FV titers in the plasma were reduced, though not to a statistically significant level. The effect of imexon on survival was not statistically significant which suggested that the antiviral effects were only transiently effective. Phytohemagglutinin-induced blastogenesis and percent of total T cells, T helper cells and T suppressor/cytotoxic cells in the spleens were increased, and the percentage of B cells decreased by imexon treatment of both FV-infected and uninfected mice. The splenic natural killer cell activity and interleukin-1 production were not markedly affected. Virus specific neutralizing antibody developed in both imexon- and placebo-treated FV-infected mice, although titers were lower in the imexon-treated animals.

Imexon; AIDS; HIV-1; Friend virus; Model; Genetically

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Introduction

Acquired immune deficiency syndrome (AIDS) is a difficult disease to treat because of its complex pathogenesis. In addition to the use of therapeutic substances targeted specifically to the replication of the virus, biological response modifiers (BRMs), specifically immunomodulators, have been the subject of considerable research efforts as an approach for enhancing current therapies (Polsky and Armstrong, 1988).

A cyanoazidine derivative, imexon (4-imino-1,3-diazobicyclo-(3.1.0)-hexan-2-one), was first described by Bicker (1978) as a BRM. It has been demonstrated to be an effective anti-cancer agent for selected tumors in mice and in humans (Bicker, 1978; Bicker et al., 1978; Micksche et al., 1978; Micksche et al., 1982). Prophylactic treatment was shown in a limited study to be effective in reducing Friend virus complex (FV)-induced splenomegaly in mice (Bicker, 1978). In this report, we describe the effect of imexon on the FV infection in a genetically defined murine model which has been recently described (Morrey et al., 1990). The model utilizes a mouse strain, (B10.A × A/WySn)F_1, that is capable of eliciting viral specific antibodies soon after retrovirus exposure despite the occurrence of immunosuppression in a manner analogous to that seen in AIDS (Morrison et al., 1986). The production and persistence of the antibodies, which are not usually seen in mouse strains not containing the Rfv-$3^{r/s}$ genotype, are correlated with a reduction of infected cells and viremia. Regardless of the virus-specific immune response, the disease is fatal.

Materials and Methods

Mice

Female B10.A and male A/WySn mice (Jackson Laboratories, Bar Harbor, ME) were mated to produce the (B10.A × A/WySn)F_1 mice. The F_1 hybrid mice were supplied as young adults weighing 18–23 g by Simonsen Laboratories (Gilroy, CA).

Virus

The Lilly-Steeves B-tropic strain of FV complex (Morrison et al., 1986) was obtained from Dr. Bruce Chesebro (NIH, NIAID, Rocky Mountain Laboratories, Hamilton) and consists of helper Friend murine leukemia virus (F-MuLV) and defective spleen focus-forming virus (SFFV). An FV stock was prepared in mice as previously described (Morrison et al., 1986).

Test compound

Imexon was provided by Boehringer Mannheim GMBIT (Indianapolis, IN) for these studies. It was stored at room temperature and made fresh each day before

treatments. Sterile saline was used as a diluent.

Viral parameters

Viral assays. Viral infectious centers (ICs) in the spleen, cell-free virus in the spleen and plasma, Northern dot blot analysis for viral RNA and spleen weights were used as indicators of viral infection. A focal immunoenzyme assay (FIEA) (personal communication, Dr. Bruce Chesebro, NIAID, NIH, Hamilton, MT) employing monoclonal antibody (mAb) 48 (Morrey and Evans, 1987; Sitbon et al., 1985) specific for envelope of Friend murine leukemia virus (F–MuLV) was used as an indication of productively infected cells and to quantitate cell-free FV in the spleen and plasma. Serial dilutions of either spleen cells, cell-free supernatant from 10^7 spleen cells/ml, or heparinized plasma were added to an 18 h monolayer of *Mus dunni* cells seeded on 24-well tissue culture plates. After cultivation for a 5-day period, confluent monolayers were incubated with mAb 48, rinsed and fixed with methanol for 5 min. After rinsing twice to remove methanol, the wells were incubated for 45 min at room temperature with peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel, West Chester, PA). A substrate, 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) in dimethyl formamide (4 mg/ml), was then added after rinsing 2–3 times with 0.01 M Tris, pH 7.6, 0.15 M NaCl, 0.002 M ethylenediaminetetraacetic acid and incubated in the dark for 20 min. Plates were rinsed with distilled water and foci counted and expressed as IC/10^6 splenocytes or focus-forming units (FFU)/ml.

Northern dot–blot hybridization. In this procedure, 5×10^6 spleen cells were lysed in a solution of vanadyl nucleoside (VRC, New England BioLabs, Beverlly, MA) as an RNase inhibitor, and Nonidet P-40 at a sufficiently low concentration to leave the nuclei intact (Meinkoth and Wahl, 1984). The nuclei were removed by centrifugation at 11 000 $\times$ *g* in a microfuge for 5 min. Cytoplasmic lysates were carefully removed and frozen at −20°C if storage was needed. After phenol/chloroform extraction, ethanol precipitation and suspension of the RNA in TE (0.01 M Tris, 5 mM EDTA, pH 8.0) containing 1 mM VRC, the RNA was denatured in 12 $\times$ SSC and 15% formaldehyde at 60°C for 15 min. The solution was blotted onto nitrocellulose (NC) paper through a dot-blot manifold 96-well template and hybridized with DNA probe specific for F-MuLV long terminal repeat (Dr. Ruth Ruprecht, Dana-Farber Cancer Institute, Boston, MA). The probe was labeled with ^{32}P-dCTP by random primer method (Boehringer Mannheim, Indianapolis, IN), using the procedure accompanying the product. After exposure of X-ray film to the NC paper, the intensity of the dots containing the samples were compared to serially diluted F-MuLV single-stranded RNA standards. The standards were made from transcription of a molecular clone of F-MuLV-57 (Evans and Morrey, 1987) in a plasmid containing a T7 RNA transcription promoter.

Immunological parameters

Splenic cell subpopulations. Subpopulations of splenic cells were enumerated with a fluorescence activated cell sorter (FACS) (EPICS-C, Coulter Corp., Hialeah, FL). Dispersed splenocytes were reacted with the following panel of fluorescein isothiocyanate- or phycoerythrin-labeled antibodies (Beckton-Dickinson, Cockeysville, MD): anti-Thy 1.2 (total T cells), anti-L3T4 (T helper cells), anti-Lyt 2 (T suppressor/cytotoxic cells), and anti-mouse IgG (total B cells).

Phytohemagglutinin-induced blastogenesis (PHA) assay. This assay was performed by pipetting 1×10^5 spleen cells into triplicate wells of flat-bottom 96-well microplates in a volume of 0.1 ml. PHA of various concentrations was added to each well in 0.1 ml aliquots and was used to monitor T cell functions. During the last 24 h of a 48 h incubation at 37°C, 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 (Warren et al., 1985). The proliferative responses were expressed as counts per minute (CPM) of [^{3}H]thymidine incorporation into splenocytes (Warren et al., 1985).

Natural killer (NK) cell activity. Splenic cells were assayed for their ability to lyse YAC-1 tumor cells in a conventional 4 h chromium release assay as an indicator of NK cell function (Warren et al., 1985). A ratio of 25 splenic cells to 1 tumor cell was used. Cytotoxicity was expressed as: % chromium release = (experimental CPM – background cpm)/(maximum cpm – background CPM).

Interleukin-1 (IL-1) assay. Splenic macrophages, separated by their adherence to plastic surface, were incubated for 24 h in 20 μg/ml of lipopolysaccharide (Morrey et al., 1990). Supernatants were removed and incubated with 10^5 U-373 cells for 48 h. After the first 24 h incubation, [^{3}H]thymidine was added to the cells; the CPM incorporation, an indication of the level of IL-1 produced by the spleen cells, was determined as with the PHA-induced blastogenesis assay. A reference standard of recombinant IL-1 (Cistron) was used to calculate the units of activity of IL-1 as follows: (CPM in test supernatants) – (CPM in media) / (CPM in IL-1 standard) – (CPM in media).

Experimental design

The three treatment schedules used were: Schedule 1, Qd × 13 beginning 1 day; Schedule 2, Qd × 11 beginning 3 days, and Schedule 3, Qd × 7 beginning 7 days after FV inoculation. Necropsy for each treatment schedule was performed at the same time after FV inoculation (day 14) to compare the parameters from each treatment schedule with the others. Ten mice were randomly assigned to each

of the treatment schedules at each dosage level, and 20 mice were assigned as placebo-treated controls to be killed on day 14. Ten uninfected mice were assigned to receive Schedule 1 at each dosage and were used as toxicity controls. Toxicity controls were not used for the other 2 treatment schedules since Experiment 1 was the longest treatment of the compound. Mice in each cage were weighed together as a group, and the difference between mean weights at the start of treatment and 24 h following final treatment were determined. To evaluate the effect of imexon on survival, 10 additional infected, imexon-treated mice, 10 uninfected, imexon-treated mice and 20 infected, placebo-treated mice were treated as described with Schedule 1 and observed daily for survival. To evaluate the effect of imexon on survival, 10 additional infected, imexon-treated mice, 10 uninfected, imexon-treated mice and 20 infected, placebo-treated mice were treated as described with Schedule 1 and observed daily for survival.

A portion of the above experiment was repeated, with FV-infected mice treated only with 100 mg/kg/day of imexon using Schedule 1. In this experiment, the disease and splenic T- and B-cell populations were monitored 35 days after virus inoculation. Neutralizing antibody titers were also determined at this time.

The data were analyzed using nested analysis of variance (Sokal and Rohlf, 1981) for comparison of mean virus titers and chi square analysis with Yates' correction used for comparison of totally negative sample numbers to placebo controls.

Results

Disease parameters

Intraperitoneal imexon treatment, 110 and 55 mg/kg/day, initiated on days 1 or 3 after FV inoculation (Schedules 1 and 2, respectively) markedly reduced the weight of spleens ($P<0.01$) of FV-infected mice as compared to placebo-treated mice (Table 1). The greatest inhibitory effect was observed in mice receiving treatment using Schedule 1. With Schedule 3, where treatment was started 7 days after viral inoculation, spleen weights were only slightly reduced ($P<0.05$) in FV-infected mice treated with the higher dosage of imexon. This splenomegaly inhibition was less pronounced after 35 days (Table 1).

Imexon treatment, while not lethally toxic at either dose used, did cause moderate host weight loss and had an effect of reducing spleen weights in toxicity control mice on day 14 (Table 1). Mean spleen weights were reduced from 84 mg in normal mice to 57 mg ($P<0.01$) and 77 mg in mice treated with 110 and 55 mg/kg/day, respectively. By day 35, this reduced spleen weight was no longer apparent (Table 1).

In mice treated with the higher imexon dosage using Schedule 1, the cell-free virus titers from spleen homogenates and FV-specific RNA/splenocyte were significantly reduced ($P<0.01$) when assayed on day 14 (Table 2). Plasma virus titers and viral infectious centers (IC)/10^6 splenocytes were reduced using Schedule 1, but the differences in the means of imexon- and placebo-treated mice were

TABLE 1
Effect of intraperitoneal imexon treatment on splenomegaly in FV-infected (B10.A × A/WySn)F_1 mice

Treatment	Schedule	Dose (mg/kg/day)	Day + 14			Day + 35	
			Toxicity controls		FV-infected	Toxicity control mean spleen wt. (mg±2 SE)	FV-infected mean spleen wt. (mg±2 SE)
			Mean wt. change (g)[a]	Mean spleen wt. (mg±2 SE)	Mean spleen wt. (mg±2 SE)		
Imexon	1	110	−0.6	57±8.26**	86±15**	73±10	829±538
		55	−0.6	77±8.26	277±91**	ND	ND
	2	110	−1.1	–	123±29**	ND	ND
		55	−0.4	–	285±86**	ND	ND
	3	110	−0.6	–	232±121*	ND	ND
		55	−0.2	–	556±311	ND	ND
Saline	1	0	–	–	510±167	–	1113±813
Normal	–	–	+1.0	84±5.11	–	71±9	–

[a]Mice were weighed as a group and the difference between mean weights at the start of treatment and 24 h following final treatment were determined.

*$P<0.05$, **$P<0.01$, compared to untreated controls.

TABLE 2

Effect of intraperitoneal imexon treatment on disease parameters in FV-infected (B10.A × A/WySn)F_1 mice

Treatment	Schedule	Dose (mg/kg/day)	Day 14				Day 35	
			Mean splenic IC[a] ($log_{10}/10^6$ splenocytes±2 SE)	Mean plasma FV (log_{10} FFU[b]/ml±2 SE)	Mean cell-free virus (log_{10} FFU/ml±2 SE)	Mean FV RNA (ng[10^{-4}]/ splenocyte±2 SE)[c]	Mean splenic IC[a] ($log_{10}/10^6$ splenocytes±2 SE)	Mean plasma FV (log_{10} FFU[b]/ml±2 SE)
Imexon	1	110	2.28 ± 0.63	1.79 ± 0.34	0.76 ± 12**	13.7 ± 2**	2.66 ± 0.58	0.97 ± 0.36
		55	3.11 ± 0.27	1.97 ± 0.56	1.51 ± 0.82**	49.5 ± 32	ND	ND
	2	110	2.90 ± 0.71	2.28 ± 0.53	2.18 ± 0.89	24.7 ± 16	ND	ND
		55	3.54 ± 0.26	2.09 ± 0.38	1.98 ± 0.77	49.5 ± 11	ND	ND
	3	110	3.44 ± 0.28	2.95 ± 0.88	1.84 ± 0.66*	55.0 ± 5	ND	ND
		55	3.33 ± 0.85	3.83 ± 1.67	–	66.0 ± 36	ND	ND
Saline	1	0	3.68 ± 0.36	2.67 ± 0.81	2.74 ±0.38	60.9 ± 28	2.42±1.16	0.70 ± 0.00
Normal	–	–	–	–	–	–	–	–

[a]Viral infectious center. [b]Focus-forming units. [c]Five samples were processed/group. *$P<0.05$, **$P<0.01$, compared to untreated controls.

not statistically significant. Delayed treatments using Schedules 2 and 3 were less efficacious in inhibiting these viral parameters (Table 2). By day 35, however, no reduction in splenic ICs or plasma virus were apparent in the imexon-treated group. These were the only viral parameters evaluated at this late sampling time.

Immunological parameters

The PHA-induced blastogenesis was suppressed in FV-infected mice when compared to uninfected mice (Table 3). This viral-induced immunosuppression was not seen, and instead, the blastogenesis was elevated to above normal values in infected mice receiving the higher dosage of imexon when treatment was initiated on day +1. When treatment was initiated using the late treatment schedules (Schedules 2 and 3), a similar, but less pronounced enhancement of PHA-induced blastogenesis again occurred (data not shown).

In uninfected toxicity control mice, imexon treatment at the higher dosage using Schedule 1 significantly ($P<0.01$) increased % of total T, T helper, and T suppressor/cytotoxic cells, but decreased % of B cells (Table 3). In the FV-infected mice, imexon at the higher dosage also had the same effect of increasing % of T cells and decreasing % of B cells using Schedule 1. A similar effect, but to a lesser degree, was seen using Schedules 2 and 3 (data not shown).

Since percentage values are a relative quantitation of splenic cell subpopulations and spleen size was altered by both FV-infection and treatment, the absolute numbers of cells/spleen were also determined (Table 4). The absolute numbers of T cells, T helper cells and T suppressor/cytotoxic cells per spleen of sham-infected mice were not significantly altered, whereas the total number of B cells/spleen were decreased ($P<0.01$) in both infected and uninfected mice receiving the higher dosage (110 mg/kg/day) of imexon.

NK cell activity, expressed as percent chromium release, was significantly ($P<0.05$) increased in FV-infected mice as compared to sham-infected mice (Table 3). Imexon treatment did not significantly alter NK cell activity in either FV-infected or sham-infected mice. This finding of increased NK activity in FV-infected (B10.A $\times$ A/WySn)F_1 mice has been confirmed in independent experiments; only in more advanced stages of disease is there observed NK depression in these FV-infected mice (unpublished results). IL-1 activity was determined not to be significantly altered by FV infection or imexon treatment (Table 3).

Both imexon- and placebo-treated, FV-infected mice had developed demonstratable neutralizing antibody titers by day 35 (Table 3), with high titers seen in the placebo-treated group.

The death pattern data of the infected, treated mice held for 160 days are shown in Fig. 1. At day +160, 40% of the placebo-control mice had survived. The infected mice treated with the 110 mg/kg/day dosage of imexon died at a greater rate than the placebo controls, with 30% having survived by day +160. The lower dosage of imexon, 55 mg/kg/day, had an opposite effect, in that greater numbers of mice (60%) survived than in the placebo-control group. The mean survival times of the three groups were: placebo-treated controls, 67 days; 55 mg/kg/day imexon-treated,

TABLE 3

Effect of intraperitoneal imexon treatment on immune parameters in FV-infected (B10.A × A/WySn)F_1 mice[a]

Group	Parameters	Day 14 mean±2 SE				Day 35 mean±2 SE		
		Imexon dosage (mg/kg/day)		Placebo	Normal	Imexon 110 mg/kg/day	Placebo	Normal
		110	55					
Toxicity controls	PHA-induced blastogenesis	8178±740*	5987±964	–	7022±746	ND	–	ND
	Natural killer cell activity[b]	22.9±4.7	15.8±2.0	–	17.2±5.1	ND	–	ND
	Interleukin-1 activity (cpm × 10^4)	44.7±10.4	41.8±8.7	–	44.5±7.5	ND	–	ND
	Splenic % total T cell	57±7**	38±4	–	35±3	44±3	–	41±2
	Splenic % T helper cell	30±3**	22±2	–	21±2	24±1	–	22±1
	Splenic % T suppressor cell	25±3**	18±2	–	16±1	17±3	–	16±1
	Splenic % B cell	26±4**	39±3	–	42±2	46±7	–	52±2
FV infected	PHA-induced blastogenesis	8033±604**	6347±791	5084±700	–	ND	ND	–
	Natural killer cell activity	25.2±2.4	28.6±2.2	28.4±3.6	–	ND	ND	–
	Interleukin-1 activity (cpm × 10^4)	43.5±8.3	40.2±4.8	47.0±6.1	–	ND	ND	–
	Splenic % total T cell	62±11**	45±3	43±4	–	60±9*	43±7	–
	Splenic % T helper cell	30±4**	23±2	20±2	–	24±2**	19±1	–
	Splenic % T suppressor cell	31±5**	22±2	21±2	–	32±5	25±3	–
	Splenic % B cell	26±7**	37±3	38±2	–	33±7**	46±3	–
	Neutralizing antibody (log_2)	ND	ND	ND	–	2.1±1.3	3.5±1.4	–

[a]qd×13 beginning on day +1. [b]% Cr-51 release assay, effector-target cell ratio of 25:1. *$P<0.05$, **$P<0.01$, compared to placebo-treated or normal controls.

TABLE 4

Effect of intraperitoneal imexon treatment on absolute cell numbers/spleen in FV-infected (B10.A × A/WySn)F_1 mice[a]

Group	Splenic cell subsets[b]	Day 14 mean cells (10^6)/spleen±2 SE				Day 35 mean cells (10^6)/spleen±2 SE		
		Imexon dosage (mg/kg/day)		Placebo	Normal	Imexon 110 mg/kg/day	Placebo	Normal
		110	55					
Toxicity controls	Total T cell	18±4	17±3	–	14±2	18±5	–	24±3
	T helper cell	7±1	8±2	–	8±2	10±3	–	13±2
	T suppressor cell	10±2	10±2	–	10±2	7±3	–	10±1
	B cell	6±2**	12±4	–	18±5	18±7	–	32±5
FV infected	Total T cell	29±14	38±9	37±7	–	17±5	13±4	–
	T helper cell	10±5	17±3	19±2	–	7±2	6±2	–
	T suppressor cell	16±8	19±3	20±3	–	9±3	8±3	–
	B cell	3±1**	22±4*	30±4	–	9±2	14±5	–

[a]qd×13 beginning on day +1.

[b]Absolute numbers of subsets of splenic lymphocytes were determined by calculating the lymphocyte subsets per total spleen. The values were calculated by total number of splenocytes, as determined by Coulter counter, times the percentage of the different lymphocyte subsets as found in Table 3.

*$P<0.05$, **$P<0.01$, compared to placebo-treated or normal controls.

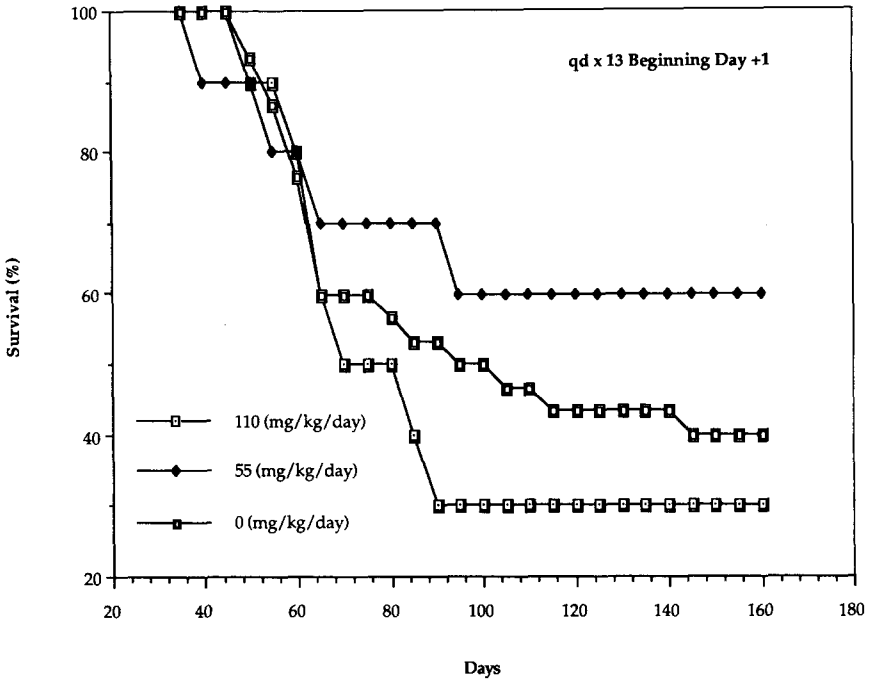

Fig. 1. Effect of intraperitoneal imexon treatment on prevention of death in FV-infected mice.

62 days, and 110 mg/kg/day imexon-treated, 68 days. Chi-square analysis with Yates' correction did not reveal any statistical significance between the survival of mice treated with 55 mg/kg/day imexon and placebo-treated mice, so it was not known if the apparent effectiveness of imexon at this dosage was biologically significant or due to normal variance.

Discussion

This study demonstrated that treatment with the BRM imexon was only moderately effective in reducing early-expressed retroviral disease parameters in this murine model. It was quite apparent, however, that after termination of treatment the disease was able to resume its course, culminating in death of the animal.

Three treatment schedules were examined in this study; that schedule utilizing imexon treatments beginning earliest after virus exposure and continuing for 13 days was most efficacious against the FV infection. Whether this enhanced efficacy was a result of the early start of treatment or was due to the prolonged number of treatments cannot be ascertained from the present studies. In somewhat similar studies with this BRM run by others in the Rauscher virus-induced murine infection, repeated 13-day therapies alternating with periods of no treatment did result in significantly increased survivor numbers (P. Black, U.S. Army Medical Research

Institute for Infectious Diseases, Frederick, MD; personal communication). Such data would suggest that it may be the prolonged number of treatments that renders the more pronounced FV disease-inhibitory effect.

The IC assay used in these experiments is an indication of the numbers of infected cells per 10^6 splenocytes. If viral-infected splenocytes were being eliminated due to a virus-specific immune response enhanced by imexon treatment, the numbers of infected splenocytes, in relation to uninfected cells, should be reduced. The mean IC/10^6 splenocytes was only slightly reduced compared to placebo controls; however, the titers of FV-specific RNA and cell-free virus in the spleen were more significantly lowered. It is possible, then, that imexon treatment did not result in the specific elimination of virus-infected cells.

Since mice from each cage were weighed together, statistical significance could not be determined, but it appeared that sublethal toxicity, apparent as whole body weight loss, was observed in control mice treated with all schedules and dosages of imexon. Imexon, referred to as BM 06 002, has been used in Phase I and II clinical studies (Micksche et al., 1978). Some patients had side effects such as vomiting and dizziness, although no adverse side effects were noticed in laboratory parameters. In none of the human cases did toxicity lead to discontinuation of treatment. In our animal studies, the weight loss seen may have been a result of either diarrhea or because the animals were in sufficient discomfort that they ate less. No pronounced diarrhea was observed in this study, so it is presumed that the weight loss was a result of lowered intake.

It has been previously reported that major host weight loss (4–7 g) alone can cause significant inhibition of splenomegaly (Sidwell et al., 1965). It is conceivable that the weight loss seen in the present study could contribute to the significant splenomegaly inhibition caused by imexon treatment, but this maximum of 1.1 g of weight loss is far less than the 4–7 g loss in the above-cited study which caused inhibition of spleen enlargement. In addition, the splenomegaly inhibition did not correlate well with loss in the toxicity control mean weight changes. It is therefore concluded that the splenomegaly inhibition in the infected, imexon-treated animals was a result of a more specific effect of the drug against the disease. In light of the previously-cited antineoplastic effects of imexon, it is possible that a similar effect occurred in this virus-induced neoplasm. The lesser inhibition of a splenomegaly on day 35 suggests the need for continued imexon treatment.

To determine the reasons for increased resistance of imexon-treated mice to FV infection, immunological parameters were assayed, but the following information about the ability of FV, and HIV-1, to infect hematopoietic cells should be realized before interpreting the data. FV does not use the same cell surface receptor as HIV-1 to attach to and infect cells, but both viruses do have the ability to infect lymphopoeitic cells of the immune system, which are the cells required for immune protection (Soldaini et al., 1989). Moreover, the transcriptional regulatory sequence (long terminal repeat (LTR)) of HIV-1, can be transactivated by certain mitogens and cytokines which results in increased production of more infectious virus (Folks et al., 1987). There is the possibility, then, that treatment with an immunomodulator may increase the number of target cells, activate lymphopoietic cells infected with

the virus or cause transactivation of the LTR which could result in increased virus production. In interpreting the immunological data, therefore, it is possible that an increase of an immune parameter may result in enhanced FV disease, or conversely, that a decrease in an immune parameter may reduce the severity of FV disease.

Imexon treatment appeared to have an effect on T cell function, since viral induced immunosuppression of PHA-induced blastogenesis was prevented. The percentage of splenic total T, T helper and T suppressor/cytotoxic cells was markedly increased in these studies, suggesting that imexon treatment may also have affected the numbers of T cells. However, when the absolute numbers of lymphocytes per total spleen were calculated, the numbers of T cells were unaltered by imexon treatment, whereas the absolute numbers of B cells/spleen were decreased. Thus, the apparent increase in relative % of T cell subsets may have been due to an absolute decrease in the numbers of B cells in the spleen. These data suggest that the imexon treatment did not enhance T cell function by increasing the absolute numbers of T cell subsets, but may have altered T cell function by increasing the function of existing cells.

The possibility that imexon treatment results in a decrease in the number of splenic B cells has an interesting implication for HIV-1 therapy. HIV-1 infection is known to cause specific B cell activation resulting in high numbers of immunoglobulin-secreting cells in the circulation (reviewed in Amador et al., 1989). This production of specific antibody might have pathogenic consequences by contributing to immune damage through antibody-dependent cell-mediated cytotoxicity of HIV-1-infected T cells (Rook et al., 1990) and/or to the generation of specific suppressor cells, which has been demonstrated in other viral infections (L'Age-Stehr et al., 1980; Tosato et al., 1979).

The observed anti-FV effects of imexon might be explained by the ability of imexon to selectively diminish numbers of splenic B cells which would serve as target cells for the virus. However, FV also infects cells of other lineages, more specifically erythroid cells (Evans and Morrey et al., 1987; Li et al., 1990). Erythroid cells are the cells that become immortalized by FV, not usually other cell types. Erythroblastosis, one of the first stages of FV-induced leukemia, is probably caused by the defective envelope protein of spleen focus-forming virus (SFFV) mimicking erythropoietin by binding to the erythropoietin cellular receptor, thereby triggering prolonged proliferation of erythroid cells, not B cells (Li et al., 1990). From these findings it is reasonable to suggest that even though B cells might be infected by FV, the infection of these cells is not required for FV-induced erythroleukemogenesis. The antiviral effects of imexon, therefore, are probably not explained by FV tropism and selective killing of B cells.

The hybrid mouse strain (B10.A $\times$ A/WySn)F_1 was used in this study because the animals contain the Rfv-$3^{r/s}$ genotype (Morrison et al., 1986). This allows them to produce significant titers of FV-specific neutralizing antibody in a similar manner to what is seen in HIV-1 diseased patients. Other, more commonly used mouse strains are incapable of developing such antibody. In both HIV-1-infected patients and in this FV-infected hybrid mouse, immunosuppression of T-cell populations occur despite the development of specific antibodies and reduction of viremia, and

virus titers in infected tissues decrease with the increased antibody titers. Despite the decreased virus titers and presence of antibody the HIV-1- and FV-infected hosts still eventually die. The hybrid mouse model has been used successfully in examining the effects of zidovudine on FV disease, with data comparing well with results found by others using other murine retrovirus models (Morrey et al., 1990). Neutralizing antibody to FV was seen in relatively high titer on day 35 in both imexon- and placebo-treated, infected mice. The antibody titers were approximately 1.5 $\log_2$ higher in the placebo-treated animals, which may have been a result of the effect of imexon on reducing the B cell populations in the mouse. In view of the possible adverse role antibody may have in HIV-1 as discussed above, this lowering of antibody titer by imexon in this study may be clinically important.

The effects of imexon on NK cell and IL-1 activity were minimal, suggesting that NK cell or IL-1 activity was not the primary affector mechanism of imexon.

Infected mice, which were treated daily for 13 days beginning one day after virus inoculation (Schedule 1), were observed for 160 days to determine effect on the usual splenomegaly-associated late-occurring death of the mice. Even though studies in this report clearly showed that this treatment markedly reduced splenomegaly as observed on day +14, only imexon treatment with the lower dosage (55 mg/kg/day) reduced the incidence of death, whereas the higher dosage (110 mg/kg/day) appeared to slightly enhance the rate of death of the infected animals. By Chi-square analysis, these differences were not statistically significant, so it was not known if the observed differences of imexon-treated mice were biologically significant as compared to those of the placebo-treated mice. The treatment schedule used may not have been optimal to cause increased survival at statistically significant levels, or imexon did not affect other pathologic factors necessary for increased survival, such as multiple steps required for immortalization of erythroid cells (Ostertag et al., 1987).

These experiments indicate that imexon, used by the treatment regimens described, is not adequate by itself to totally inhibit this retrovirus infection in mice. The material has obvious immunomodulatory and probable antineoplastic properties which may be of value, particularly when used in combination with a more specific retrovirus-inhibiting drug. Experiments examining such drug combinations in experimental retrovirus models are underway in several laboratories.

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