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HOST RESISTANCE MODELS AVAILABLE FOR IMMUNOTOXICOLOGIC STUDIES

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INTRODUCTION

For about 25 years various investigators at IIT Research Institute (IITRI) have been developing animal model systems to measure the holistic response to drugs, chemicals, and air pollutants. In 1958, the first report on the effects of ozone (O_3) on bacterial pneumonia was published by Miller and Ehrlich. That research, supported by the United States Air Force, was the basis for our subsequent programs in which several infectious disease models were used to evaluate the effects of a variety of toxicants. Table 1 shows thirteen infectious disease models and one tumor challenge model we have used in mice, hamsters, guinea pigs, or squirrel monkeys. With the exception of the diethylstilbestrol (DES), all toxicants were administered by the inhalation route. Other investigators are working with a variety of tumor and infectious disease models to evaluate toxicants administered by various routes and Dean et al. (1982b) have summarized this research. As a consequence there is a wide selection of host resistance models available for use.

Recently, increasing attention has been focused on the two-directional aspect of immune modulation following exposure to the various test agents. First, it has been aimed towards detection and prevention of adverse effects of agents on host immunity, with a view towards avoiding a resulting increased susceptibility to infectious disease or to a malignant process. Secondly, attention has been directed toward the stimulation of host resistance to certain disease entities.

To develop an immunologic profile, the selection of the appropriate assay system (Table 2) is dependent upon which aspect of immune modulation is being studied, i.e., suppression or stimulation; reproducibility of the disease state; susceptibility of a given host to a particular disease; easily measured parameters; route of exposure to both the infectious organism/tumor system and test material; and the cost. Host resistance assays are important since they reflect in the host the net result of alterations in specific immune aspects that may be detected separately by predominantly in vitro assays.

TABLE 1. ANIMAL MODEL SYSTEMS ESTABLISHED AT IITRI

Toxicant	Organism	Animal Model	Reference
O_3	Klebsiella	Mice, hamsters	Miller & Ehrlich 1958
NO_2	Klebsiella Klebsiella	Mice Squirrel monkeys	Ehrlich, 1963 Ehrlich, 1966
Cigarette Smoke	Klebsiella Staphylococcus Diplococcus Influenza	Mice	Spurgash et al., 1968
NO_2	Klebsiella Influenza	Squirrel monkeys	Henry et al., 1970a
NO_2 and Cigarette Smoke	Klebsiella	Hamsters	Henry et al., 1970b
Hyperoxia	Influenza	Mice	Fenters et al., 1975a
PtO_2 and PdO	Klebsiella Influenza Streptococcus	Mice	Fenters et al., 1975b
MnO_2	Klebsiella Influenza	Mice	Maigetter et al., 1976
NO_2/O_3 Mixtures	Streptococcus	Mice	Ehrlich et al., 1977
Sulfates	Streptococcus	Mice	Ehrlich et al., 1978
SO_2 and Nitrates	Streptococcus	Mice	Ehrlich, 1979
Acid mists	Influenza	Mice	Fenters et al., 1979
O_3	Mycobacterium	Mice, guinea pigs	Thomas et al., 1981a
PAN	Streptococcus Mycobacterium	Mice	Thomas et al., 1981b
Industrial Particulates	Streptococcus	Mice	Aranyi et al., 1981a
As_2O_3	Streptococcus	Mice	Aranyi et al., 1981b
DES	Listeria, Herpes, Trichinella, Influenza, Streptococcus B16-F10 melanoma	Mice	Bradof et al., 1982

TABLE 2. SELECTION OF HOST RESISTANCE MODEL

- Immunomodulation
  - Suppression
  - Stimulation
- Cost
- Reproducibility of Disease State
- Susceptibility to Disease
- Easily Measured Parameters
- Route of Exposure
  - Test Agent
  - Test Material

This paper stresses the importance of selecting the appropriate assay model for a given test agent with references to the host resistance assay panel currently used in our laboratories to screen for alterations in host immunity. Data representative of several kinds of toxicants administered by various routes are presented. These studies are being supported by the U.S. Environmental Protection Agency and the National Institute of Environmental Health Sciences.

METHODS

In studies conducted at IITRI, male and female CD-1 or female B6C3F1 mice were obtained commercially and used when 5 to 8 weeks old. Animals were housed 5 to 10 per cage and received laboratory chow and water ad libitum.

DES ADMINISTRATION

Varying concentrations of the synthetic estrogen DES were injected subcutaneously (sc) in 0.05 ml of corn oil for 14 consecutive days. After 2 to 3 days rest, mice were challenged with an infectious agent or with tumor cells. Details of exposure to other toxicants are appropriately referenced.

HOST RESISTANCE ASSAY PANEL

INFECTIOUS AGENTS

- Listeria monocytogenes was prepared as frozen (-70°C) stock cultures. A modification of techniques described by Dean et al. (1980) and Newborg and North (1980) was used. A cell concentration

of approximately 3 x 10^6 colony forming units (CFU) was used to result in a lethal dose of 10 to 30% (LD_{10-30}) in control mice. Animals were monitored daily for 10 days for death.

- Group C Streptococcus sp., obtained from EPA, was prepared as described by Aranyi et al. (1981a). For intraperitoneal (ip) challenge, approximately 2 x 10^5 CFU of the streptococcal suspension provided an LD_{10-30}. The mice were observed for deaths over a 10-day period. For respiratory challenge, mice exposed to the toxicant and control mice were treated simultaneously with a bacterial aerosol that was generated with a model 841 DeVilbiss nebulizer in a Plexiglas chamber. The 432-liter main compartment is suitable for exposure of 180 mice confined in individual wire cages. Details were described by Ehrlich et al. (1977) and Aranyi et al. (1981a). Mice were observed for 14 days for mortality.

- Influenza A_2/Taiwan/64 virus aerosol challenge procedures were described by Fenters et al. (1979). Animals were observed for 14 days after infectious challenge. Survivors were killed and the extent of pulmonary lesions was expressed as a percentage of total lung consolidation (Horsfall, 1939).

- Herpes simplex virus (HSV) challenge was performed using both Type 1 (S-148 Nahmias strain) and Type 2 (ATCC VR 540). The HSV 1 virus, obtained as a mouse-adapted strain from Schering Corporation, was passaged two times through rabbit kidney cells. The HSV 2 virus was passed once through L929 mouse cells and two times through rabbit kidney cells. In each case, the supernatants were harvested at 3 to 4+ cytopathogenicity (CPE), aliquoted, and frozen away at -70°C. For use, the virus was diluted in phosphate buffered saline (PBS) and an LD_{20-30} dose in 0.2 ml was injected ip.

- Trichinella spiralis infection was performed using modified methods of Larsh and Kent (1949) and Weatherly (1970). Mice were infected by gavage with 0.2 ml aliquots containing approximately 200 larvae. Groups of infected, non-treated control mice were included in all experiments and were killed at 7 days post-infection for adult worm counts (viability control). Experimental and control mice were sacrificed 14 days after infection, the number of adult worms remaining in the small intestine was enumerated microscopically, and the average of three consecutive counts from each mouse was determined.

TUMOR SUSCEPTIBILITY ASSAY

- The B16-F10 melanoma tumor cell line of Fidler (1973; 1975) was obtained from the NCI-Tumor Bank and the procedures followed were essentially the same as he described. Cells were harvested from cultures in the exponential growth phase, pooled, and counted

on a Coulter counter. The cell suspension was adjusted to 2.5×10^5 cells/ml with HBSS and 0.2 ml was injected intravenously (iv) into mice within 15 minutes of cell preparation. Mice were sacrificed 3 weeks later, and the lungs were placed into cold water and weighed individually on an analytical balance.

STATISTICAL ANALYSES

At IITRI, three methods were employed for statistical analysis. The mixed-model analysis of variance (ANOVA) as described by Winer (1975) was chosen for continuous response data. In addition to the univariate analyses, multivariate test statistics (Anderson, 1958; Bock, 1975) also were included when there was theoretical justification to believe that simultaneous responses in several biological systems were interrelated. A log-linear model, appropriate for discrete or qualitative response data, was used to evaluate mortality data (Bishop et al., 1975; Haberman, 1979). For between group comparison of survival time, the product limit estimator (Kaplan and Meier, 1958) was used in conjunction with the Mantel-Cox test statistic (Cox, 1972).

RESULTS AND DISCUSSION

• Listeria monocytogenes

This assay system exemplifies a model that may be utilized to detect either increased or decreased host resistance. Listeriosis is regarded as a zoonosis, with a wide range of clinical manifestations ranging from meningitis to urethritis in humans. The pathogenesis is well documented and Newborg and North (1980) have shown that the immune response to Listeria infection is regulated primarily by immunocompetent T-cells and macrophages. This is noteworthy in that some bacterial models evoke primary mechanisms of resistance which are non-specific, e.g., polymorphonuclear leukocyte phagocytosis.

The two parameters measured in this assay were mortality and survival time. Replicate experiments with DES are shown in Table 3. The results show that mortality was increased significantly and mean survival time was decreased in all DES-treated groups relative to controls. These data are similar to those reported by Dean et al. (1980). Note that DES in excess of 0.2 mg/kg was associated with decreasing mortality. Statistical analysis showed that there was a significant quadratic dose-response relationship and the effect was due to a decrease in mortality in the high dosage group in both replicate experiments. This decrease in mortality associated with increasing concentrations of DES may reflect macrophage activation. Boorman et al. (1980) have shown that DES exposure increases both macrophage numbers and activation.

TABLE 3. EFFECT OF DES ON RESISTANCE OF FEMALE B6C3F1 MICE TO LISTERIA MONOCYTOGENES CHALLENGE

DES mg/kg	Mortality D/T[b]	Mortality %	MST[a] (Days)	Number of Replicate Experiments
0	4/20	20	8.8	2
0.2	19/20	95[c]	4.3[c]	
1.0	18/20	90[c]	4.7[c]	
4.0	15/20	75[c]	5.6[d,e]	

a Mean survival time
b Deaths per total animals
c Difference from control: $p \leq 0.0001$
Quadratic dose-response relationship: $p < 0.0001$
d Difference from control: $p \leq 0.001$
e Difference from 0.2 mg/kg: $p \leq 0.06$

In another study (Table 4), replicate experiments were conducted with cadmium chloride ($CdCl_2$). Mice received 14 daily intragastric (ig) injections of sterile pyrogen-free water containing the $CdCl_2$. In contrast to the DES data, no effects were noted. However, the control mortality (78%) was high and it would be difficult to detect an increase in susceptibility to infection. On the other hand, high control values are required to discern immunopotentiation. In a preliminary study with a maleic anhydride-vinyl copolymer (MVE-2), the agent was given iv at 20 µg/day for 5 days before challenge with Listeria. As shown in Table 5, host resistance was enhanced. The control mortality was 90% and mean survival time was 3.0 days. The experimental group had a 30% mortality and a survival time of 7.6 days. A replicate study has been completed and similar data were obtained.

TABLE 4. EFFECT OF $CdCl_2$ ON RESISTANCE OF FEMALE B6C3F1 MICE TO LISTERIA MONOCYTOGENES CHALLENGE

$CdCl_2$ mg/kg	Mortality D/T	Mortality %	MST[a] (Days)	Number of Replicate Experiments
0	43/55	78	4.8	3
0.4	31/37	84	4.6	
2.0	48/53	91	3.7	
4.0	48/54	89	4.2	
8.0	12/18	67	5.4	

a Mean survival time

TABLE 5. IMMUNOSTIMULANT EFFECT OF MVE-2 IN LISTERIA MONOCYTOGENES CHALLENGED FEMALE CD-1 MICE

MVE-2 µg/mouse	Mortality D/T	Mortality %	MST[a] (Days)
0	(9/10)	90	3.0
100	(3/10)	30	7.6

[a] Mean survival time

Thus the Listeria model shows intra- and interlaboratory reproducibility, requires few animals, and is capable of reflecting immunopotentiation as well as immunosuppression.

- **Streptococcus**

This model stresses the importance of the route of administration of both test and challenge agents. Specific immunity against streptococcal infections is not well defined. However, there is evidence suggesting that specific antibody as well as alveolar macrophages and other non-specific phagocytic cells are important in host protection.

INTRAPERITONEAL CHALLENGE

The DES-treated mice were challenged ip with streptococcus and observed for 10 days for mortality and survival time (Table 6). No significant differences in mortality or in survival time between control and DES-treated mice were detected. In another study (Table 7) 80 mg/kg of cyclophosphamide (CY), a well known immunosuppressant, was injected ip either 1 day prior to, or the same day as, ip challenge with streptococcus. Again, there was no difference in mortality when compared to controls.

TABLE 6. EFFECT OF DES ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO STREPTOCOCCUS sp. INTRAPERITONEAL CHALLENGE

DES mg/kg	Mortality D/T	Mortality %	MST[a] (Days)
0	2/21	10	9.5
0.2	3/19	16	9.3
1.0	1/20	5	9.8
4.0	4/18	22	9.1

[a] Mean survival time

TABLE 7. EFFECT OF CY ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO STREPTOCOCCUS sp. INTRAPERITONEAL CHALLENGE

CY mg/kg	Mortality D/T	Mortality %	MST[a] (Days)
0	5/20	25	9.0
80[b]	4/20	20	9.3
80[c]	3/20	15	9.4

[a] Mean survival time
[b] 1 day prior to challenge with streptococcus
[c] 4 hours prior to challenge with streptococcus

AEROSOL CHALLENGE

The results of three replicate experiments in which mice were treated with DES for 14 days, challenged with airborne streptococcus, and observed for 14 days are summarized in Table 8. Analysis of the results showed no significant effect of DES treatment on mortality. However, when mice were injected with 8 mg/kg DES on Days 1 through 5 and 8 through 12, a significant increase in mortality occurred in the DES-treated mice compared with controls. In addition, survival times were found to be significantly decreased following the high dose of DES.

TABLE 8. EFFECT OF DES ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO STREPTOCOCCUS sp. AEROSOL CHALLENGE

DES mg/kg	Mortality D/T	Mortality %	MST[a] (Days)	Number of Replicate Experiments
0[b]	15/68	22.1	12.1	3
0.2	13/73	17.8	12.6	
1.0	20/71	28.2	11.5	
4.0	21/68	30.9	11.6	
0[c]	12/67	17.9	12.3	3
8.0	29/69	42.0[d]	10.9[d]	

[a] Mean survival time
[b] sc injections for 14 days
[c] sc injections Days 1 through 5 and 8 through 12
[d] $p < 0.01$ (Mortality: log linear model; MST: Mantel-Cox test)

When the DES and CY were administered systemically, no changes were found in susceptibility to the respiratory agent, streptococcus, except at one high DES concentration. However, if the route of exposure to a toxicant is inhalation, this host resistance model has been shown to be quite sensitive. In studies reported by Ehrlich et

al. (1978) the relative toxicity of zinc sulfate and ammonium sulfate was assessed using this streptococcal model. Mice inhaled varying concentrations of the sulfates for 3 hours and immediately thereafter were challenged with airborne streptococcus. The data in Table 9 show that inhalation of 1.2 mg/m^3 of zinc sulfate resulted in significant increases in mortality and reduced survival time. In contrast, inhalation of ammonium sulfate, which has been shown to be relatively non-toxic in other assays (unpublished data), had little or no effect on this respiratory infection in concentrations up to 5.3 mg/m^3. The large number of mice required for the aerosol studies should be noted.

TABLE 9. MORTALITY AND SURVIVAL RATE OF CD-1 FEMALE MICE EXPOSED FOR 3 HOURS TO SULFATES AND CHALLENGED WITH STREPTOCOCCUS AEROSOL

SO_4 mg/m^3	$ZnSO_4$ Mortality D/T	$ZnSO_4$ Mortality %	$ZnSO_4$ MST[a] (Days)	$(NH_4)_2SO_4$ Mortality D/T	$(NH_4)_2SO_4$ Mortality %	$(NH_4)_2SO_4$ MST[a] (Days)
0	373/1689	22.1	12.1	233/588	39.6	10.1
≤1.1	125/599	20.9	12.2	22/48	45.8	10.1
1.2-2.0	369/813	45.4[b]	9.1[b]	76/191	39.8	10.7
2.1-3.0	186/278	66.9[b]	6.2[b]	52/96	54.2[b]	9.2
3.1-4.0	---	---	---	47/144	32.6	11.2
≥4.1	---	---	---	46/110	41.8	10.3

[a] Mean survival time

[b] Significant difference from control determined by Chi-square test (mortality) or Student's t test (MST): $p \leq 0.05$.

Data from Aranyi et al. (1981a) further illustrate the usefulness of the streptococcal model in differentiating degrees of toxicity (Table 10). Multiple exposure of mice to 2 mg/m^3 of copper smelter dust resulted in greatly increased mortality whereas no effects were seen in mice exposed to coal fly ash. These investigators also found significantly depressed pulmonary bactericidal activity in mice exposed to the copper smelter dust, whereas no changes in bactericidal activity were found in mice exposed to the fly ash.

Similar host resistance studies with airborne streptococcus were conducted in our laboratory and at the EPA. Exposure to various inhalation hazards such as nitrogen dioxide and/or ozone (Ehrlich et al., 1977; Ehrlich, 1979 and 1980; Gardner et al., 1977a; Gardner, 1980), or aerosols such as sulfate salts (Ehrlich, 1980; Ehrlich et al., 1978), sulfuric acid (Gardner et al., 1977a), cadmium chloride (Gardner et al., 1977b), and arsenic trioxide (Aranyi et al., 1981b), also demonstrated the sensitivity of this assay to inhaled toxicants.

TABLE 10. EFFECTS OF MULTIPLE DAILY 3-HOUR EXPOSURES TO AEROSOLS OF COPPER SMELTER DUST OR COAL FLY ASH ON SUSCEPTIBILITY TO RESPIRATORY STREPTOCOCCUS INFECTION IN CD-1 FEMALE MICE

Aerosol Exposure				Streptococcus Infection		
	Conc., $\mu g/m^3$		No. of Exposures,		%	MST[a]
Sample	Mean	S.D.	3 hr/day	n[b]	Mortality	(Days)
Copper Smelter Dust	2050	190	5	166	33.6***	-2.5***
			10	216	27.2***	-2.9***
			20	268	19.0***	-1.3**
Coal Fly Ash No. 2 (Fluidized-bed)	2040	370	5	325	0.1	-0.2
			10	276	-3.4	0.1
			20	299	1.6	-0.1

[a] Mean survival time
[b] Number of mice
Significant change from corresponding control mice:
** $p \leq 0.05$ *** $p \leq 0.001$

● **Influenza virus** is the second inhalation model demonstrating an effect of route of administration. Humoral immunity and interferon production are the main defenses against influenza infection. Studies (Table 11) with DES, administered by the systemic route, showed no effect on the host's resistance to airborne influenza virus challenge as measured by mortality, survival time, or pulmonary consolidation scores.

TABLE 11. EFFECT OF DES ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO INFLUENZA A_2/TAIWAN/64 AEROSOL CHALLENGE

DES	Mortality		MST[a] (Days)		Pulmonary Consolidation		Number of Replicate
mg/kg	D/T	%	Mean	± S.E.	Mean	± S.E.	Experiments
0	18/47	38.3	12.77	0.77	3.65	0.16	2
0.2	13/48	27.1	13.21	0.70	3.81	0.17	
1.0	18/48	37.5	12.67	0.91	3.65	0.15	
4.0	15/48	31.3	12.87	0.67	3.77	0.18	

[a] Mean survival time

However, as with our streptococcal model, when the toxic test material also was delivered by the inhalation route, an effect was noted with the inhaled respiratory virus. In previous studies in our laboratories (Fenters et al., 1979) male CD-1 mice were exposed 3 hr/day, 5 days/week for up to 20 weeks to respirable-size carbon particles (1.5 mg/m^3) with sulfuric acid (1.4 mg/m^3) adsorbed on

their surface. Data in Table 12 show no effects on mice exposed for 4 weeks. After 20 weeks, however, changes could be detected in mice exposed to acid-coated carbon compared to air or carbon controls. Again, this test system using airborne infectious challenge required a large number of mice.

TABLE 12. LONG-TERM EXPOSURE TO ACID MISTS: RESPONSE OF CD-1 MALE MICE CHALLENGED WITH INFLUENZA A_2/TAIWAN VIRUS

Duration of Exposure (wk)	Experimental Condition	Mortality[a] D/T	Mortality[a] %	MST[b] (Days)	Pulmonary Consolidation
4	Air	16/164	10	13/5	2.05
	Carbon	10/164	6	13.7	1.62
	Acid/Carbon	13/166	8	13.7	1.70
20	Air	48/133	36	12.2	3.11
	Carbon	46/137	34	12.3	3.07
	Acid/Carbon	61/136	45*	11.7**	3.43**

a Chi-square test
b Mean survival time: Student's t test
* Significant difference ($p \leq 0.10$) compared to carbon control
** Significant difference ($p \leq 0.10$) compared to both air control and carbon

In summary, our studies of airborne bacterial and viral infections have emphasized the importance of the route of administration of the test material. However, they also show that the requirement for fairly large numbers of animals and for costly sophisticated laboratory equipment and engineering controls often obviate the utilization of these models in many laboratories. On the other hand, these host resistance assays are highly sensitive and correlate well with other parameters, such as the pulmonary bactericidal activity, when measuring effects of airborne toxicants. Also, these assays are reproducible between laboratories as attested to by results obtained at IITRI and EPA using the streptococcal model. However, the variation within replicate aerosol assays often is greater than when the infectious challenge is by the systemic route.

- **Herpes simplex virus (HSV)** is another useful model system which has systemic tropism. Cellular immunity, macrophages, and interferon are involved in resistance to herpes virus and it is well documented that members of the herpes virus group cause severe illness in immunocompromised people. Kern (1982) reports that this viral model has been used for detecting immunosuppression as well as immunopotentiation. We have used the herpes mouse model for more than 10 years in our antiviral testing program and we, too, find immunostimulation with compounds from industrial clients. We have used both HSV 1 and 2 since it is not clearly understood if the immune response to these two "different" types is similar.

In our system mice are inoculated ip, allowing the virus to first be detected in the gut, spleen, and liver and ultimately in lung, brain, and spinal cord. Thus there is a generalized infection. In studies with DES (Table 13) there are significant increases in mortality and decreases in survival time between control and DES-treated mice that were infected with HSV 1. Similar increases in mortality were reported by Kern (1982) in mice treated with CY and infected with HSV 1. Data in Table 14, representing HSV 2, are similar. In both cases, the DES significantly suppressed host resistance.

TABLE 13. EFFECT OF DES ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO INFECTION WITH HERPES SIMPLEX VIRUS, TYPE 1

DES mg/kg	Mortality D/T	Mortality %	MST[a] (Days)	Number of Replicate Experiments
0	5/48	10.4	19.8	2
0.2	14/49	28.6[b]	17.2[b]	
1.0	17/48	35.4[d]	16.7[c]	
4.0	16/44	36.4[d]	16.5[c]	

Difference from control determined by Mantel-Cox test (MST) or by log linear analysis (mortality):

[a] Mean survival time
[b] $p < 0.05$
[c] $p < 0.01$
[d] $p < 0.001$

TABLE 14. EFFECT OF DES ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO INFECTION WITH HERPES SIMPLEX VIRUS, TYPE 2

DES mg/kg	Mortality D/T	Mortality %	MST[a] (Days)	Number of Replicate Experiments
0	8/70	11.4	19.8	4
0.2	12/69	17.4	19.1	
1.0	17/62	27.4[b]	17.1[c]	
4.0	19/66	28.8[b]	17.7[c]	

Difference from control determined by Mantel-Cox test (MST) or by log linear analysis (mortality):

[a] Mean survival time
[b] $p < 0.05$
[c] $p < 0.01$

A neglected area of research is the effect of toxic agents on the secondary immune response. We recently initiated studies with cadmium chloride and evaluated both primary and secondary immune responses to HSV 1. As Table 15 shows, with primary virus challenge the control and experimental mortality rates were similar and, as expected, complete protection from death was seen after the secondary challenge. Thus $CdCl_2$ treatment when administered before the primary challenge did not interfere with the development of a strong immune response.

TABLE 15. EFFECT OF $CdCl_2$ ON SUSCEPTIBILITY OF B6C3F1 FEMALE MICE TO PRIMARY AND SECONDARY INFECTION WITH HERPES SIMPLEX VIRUS, TYPE 1

$CdCl_2$ mg/kg	Primary Challenge[a] Mortality D/T	Mortality %	MST[c] (Days)	Secondary Challenge[b] Mortality D/T	Mortality %	MST[c] (Days)
0(1°)	5/20	25.0	17.8	0/15	0***	21.0***
0.4	2/26	7.7	19.9	0/24	0***	21.0***
2.0	4/18	22.2	18.5	0/14	0***	21.0***
4.0	3/18	16.7	18.6	0/15	0***	21.0***
0(2°)	----	----	----	11/20	55.0	14.2

[a] $CdCl_2$ was given ig for 12 days: 3 days later mice were challenged ip with herpes virus

[b] Secondary challenge with virus was administered 21 days after the primary challenge to the surviving mice and to an additional group of distilled water-treated mice which served as control for the second challenge

[c] Mean survival time

Significant difference from control determined by Chi-square test (mortality) or Student's t test (MST):

***$p \leq 0.001$

The questions remain

- what would be the effect on the secondary response when using a known immunosuppressant such as CY or DES before primary challenge?
- what effect would occur on the secondary response when an immunosuppressant or immunopotentiator was given after the primary microbial or tumor challenge? Such studies are in progress in our laboratories.

Thus the herpes model gives reproducible data, requires relatively small numbers of mice, and is capable of evaluating suppression as well as potentiation of the immune response.

• EXPULSION OF TRICHINELLA SPIRALIS

Delaved hypersensitivity and cellular immunity are important in defending a host against T. spiralis (Larsh et al., 1974). Immunity to T. spiralis larvae rids infected mice of the adult parasite through expulsion from the gut in about 14 days. Thus the presence of an increased number of adult parasites at Day 14 indicates impaired host resistance and immunologic changes.

Studies by Dean et al. (1980) showed that a concentration as low as 0.2 mg/kg of DES suppressed the immune system sufficiently to allow significant increases in the number of adult worms. We obtained similar results as shown in Table 16. This assay, as with Listeria, is so sensitive that as few as 10 mice can be used to obtain reproducible data for interlaboratory validation. Unlike Listeria, we have seen no published reports showing the usefulness of this model system to study immunopotentiation.

TABLE 16. EFFECTS FROM DES ON EXPULSION OF ADULT TRICHINELLA SPIRALIS FROM B6C3F1 FEMALE MICE

DES mg/kg	N	Average Number of Adult Worms at Day 14	% Expulsion Relative to Controls at Day 7
0	10	0	100
0.2	10	0	100
1.0	10	2.9[a]	89.3[a]
4.0	10	11.3[a]	58.2[a]

[a] $p \leq 0.0001$ (Mixed model ANOVA)

TUMOR MODELS

As stated earlier, care should be exerted in the selection of a given model dependent upon the agent being tested, the route of exposure to the agent, and the flexibility of the model for measuring stimulatory as well as adverse effects on host resistance. To illustrate this point, we have selected three tumor models (Table 17) for examination: the 4198T tumor line, a polyoma virus-induced tumor line developed by Dr. Robert Ting; the PYB6 sarcoma, a polyoma virus-induced tumor line; and the B16-F10 melanoma tumor line of Dr. I. J. Fidler, a metastatic line developed for the ability to form pulmonary tumor foci.

TABLE 17. CONSIDERATIONS FOR SELECTING TUMOR SUSCEPTIBILITY MODELS

Tumor	Site of Implantation	Useful for Measuring: Decreased Host Resistance	Useful for Measuring: Increased Host Resistance	Endpoint Measurement
4198T	sc	maybe	no	Mortality, tumor incidence or growth rate
PYB6	sc	yes	maybe	Mortality, tumor incidence or growth rate
B16-F10	iv	yes	yes	Number of tumor foci, lung weights, IUDR incorporation

The 4198T tumor cell line requires approximately 10^6 cells/mouse, injected sc, to produce a tumor incidence of only 10 to 20% within 60 days. In addition to requiring this relatively large number of cells and the expense of growing sufficient cells in culture, the model effectively can measure only decreased host resistance since too many cells would be required to produce a 70-80% tumor incidence. In fact, this model has not been found particularly satisfactory even for measuring decreased resistance (Dean, personal communication).

The PYB6 tumor requires only about 5×10^3 cells sc to produce a 10 to 40% incidence and this model has been utilized to demonstrate decreased host resistance from exposure to a number of agents including DES (Dean et al., 1982a). Although it should be useful for detecting increased host resistance, we have seen no reports to that effect as a result of exposure to any test agent.

In contrast, the B16-F10 melanoma cell model, which requires about 5×10^4 cells/mouse, has been shown to be sensitive in detecting decreased host resistance using CY and the phorbol ester TPA (Dean et al., 1982a) as well as increased host resistance (Table 18). In our study, 3 weeks after iv challenge with 5×10^4 B16-F10 melanoma cells, the mice were killed and lungs removed. In two replicate experiments, this concentration of tumor cells resulted in lung tumor foci too numerous to count and a statistically significant reduction of tumor mass in the lungs of all the DES-treated animals as compared with the controls. These data contrast with the findings of Dean et al. (1982a) with DES and the PYB6 model. A possible explanation is that B16 melanoma cells are highly sensitive to activated macrophages (Fidler, 1974) and DES is reported to be a potent macrophage activator (Boorman et al., 1980). Whether this activation is reflected as cytostasis or is cytocidal remains to be determined and indicates further potential usefulness for the B16-

F10 model in helping delineate mechanisms of action of certain agents. Finally, because the lungs are the site of tumor growth, this model should be particularly useful in evaluating effects of inhaled toxicants.

TABLE 18. EFFECT OF DES ON TUMOR GROWTH IN THE LUNGS OF B6C3F1 FEMALE MICE FOLLOWING IV INOCULATION OF 5 X 10^4 B16-F10 MELANOMA CELLS

DES mg/kg	N	Lung Weights (mgs) Mean	± S.D.	Number of Replicate Experiments
0	20	824	476	2
0.2	20	530[a]	427	
1.0	20	553[a]	360	
4.0	19	523[a]	282	

[a] $p \leq 0.004$ (Mixed model ANOVA)

The uses of tumor models in immunotoxicology will be more extensively discussed by Dr. Kerkvliet in the following paper.

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