

## IN VITRO CELL-MEDIATED IMMUNITY STUDIES OF PLUTONIUM-EXPOSED BEAGLE DOGS

### Investigators:

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Mitogen-induced activation was measured in spleen and mesenteric lymph node cell preparations from dogs exposed to a single inhalation exposure of plutonium oxide ( $^{238}\text{Pu}$  or  $^{239}\text{Pu}$ ). Reduced stimulation indices of splenic lymphocytes from exposed animals suggest that a reduction in lymphocyte function has occurred in this tissue. No apparent reduction in mitogen stimulation indices was observed in mesenteric lymph node cultures.

Previous studies on the biological effects of inhaled  $^{239}\text{PuO}_2$  or  $^{238}\text{PuO}_2$  in beagle dogs have indicated that the most significant effects were the appearance of chronic lymphocytopenia, respiratory insufficiency, and pulmonary neoplasia after initial alveolar deposition of 0.2 to 3.0  $\mu\text{Ci}$ . In these studies, lymphocytopenia persisted throughout the life span of the animals and was often the only observed effect of the single plutonium exposure. Subsequent studies have established that the lymphocytes in peripheral blood of plutonium-exposed dogs were not only reduced in number per unit volume of blood but also in functional capacity, as monitored with mitogen stimulation studies (Annual Report, 1979).

To determine if the loss in mitogen responsiveness of lymphocytes had occurred in other lymphoid tissues, mitogen studies have been conducted on spleen and mesenteric lymph node cells obtained from long-term plutonium-exposed dogs at sacrifice.

The assay system used in these studies is outlined in Figure 74. Basically, spleen and mesenteric lymph nodes of dogs were removed aseptically. Cell suspensions of the tissues were prepared, total and differential counts were made, and viability was checked with trypan blue. The cells were diluted with supplemented RPMI 1640 tissue culture media (containing 10% heat-inactivated fetal bovine serum) to give a final density of  $2.2 \times 10^6$  viable cells per ml in the assay.

The mitogens used were: Concanavalin-A (Con-A) and phytohemagglutinin (PHA), plant lectins that stimulate T lymphocytes; lipopolysaccharide (LPS, a B-cell-activating endotoxin); and pokeweed mitogen (PWM), a plant lectin, mainly specific for B lympho-

cytes, but which also stimulates a small portion of the T-cell population.

Lymphocyte activation was estimated by measuring the incorporation of  $^{125}\text{I}$ -iododeoxyuridine ( $^{125}\text{I}$ -IUDR) into newly synthesized DNA. The extent of  $^{125}\text{I}$ -IUDR incorporated by lymphocytes was measured. The data are reported as a stimulation index (SI); after 3 days of cell incubation in the presence of the mitogens at selected concentrations, obtained by dividing the total cpm of  $^{125}\text{I}$ -IUDR incorporated by stimulated cultures by the total  $^{125}\text{I}$ -IUDR incorporated by unstimulated lymphocytes.

In spleen culture studies (Table 68), we have observed a reduced mitogen response with lymphocytes from severely lymphopenic dogs. Specifically, the SI of splenic lymphocytes from  $^{238}\text{PuO}_2$ -exposed dogs showed reduced mitogen stimulation which is related to both the initial deep lung deposition of plutonium oxide and the presence of bone tumors. The SI of two low-level  $^{238}\text{Pu}$ -exposed dogs (#1189, #1312), with no diagnosed tumors, was high. In  $^{239}\text{PuO}_2$ -exposed dogs, the Con-A-induced stimulation of spleen lymphocytes was consistently repressed and correlated with the presence of lung tumors. However, apparent correlation was observed between SIs of spleen lymphocytes and initial deep lung depositions of  $^{239}\text{PuO}_2$ -exposed dogs. Thus far, we have analyzed the spleen data from one control animal, which was 10 yr old at death. It should be noted that we do not know the specific effects that aging may have on immune functions estimated by mitogen stimulation assays.

Relatively high and variable SIs have been observed with mesenteric lymph node cells from exposed dogs (Table 69). No

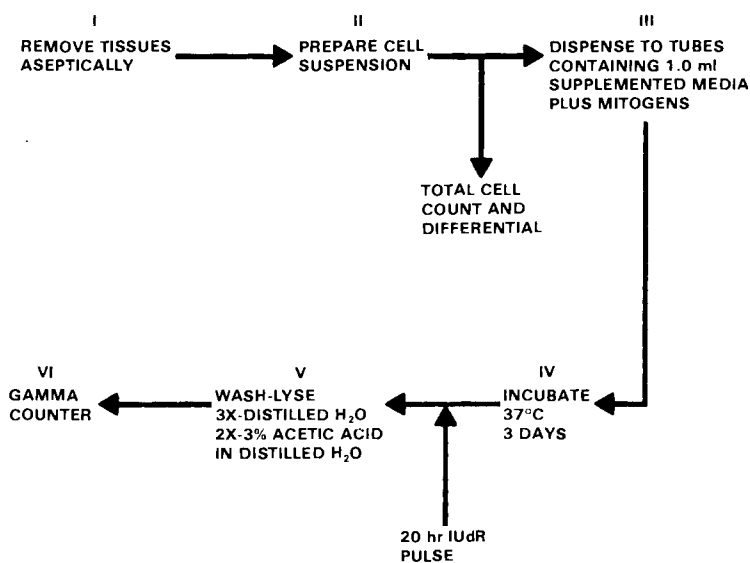


FIGURE 74. Flow Diagram of Tissue-Derived Lymphocyte Activation Studies

TABLE 68. Mitogen Stimulation of Spleen Lymphocytes from Control and Exposed Beagle Dogs

Dog No.	Sex	Age, yr	Years After Exposure	Inhaled Plutonium Oxide	Initial Alveolar Deposition, $\mu\text{Ci}$	Tumor	Mitogen Stimulation Index <sup>(a)</sup> $\pm$ SD			
							Con-A	PHA	PWM	LPS
1037	M	7.4	5.8	$^{238}\text{Pu}$	4.854	Bone	$1.7 \pm 0.5$	$2.0 \pm 0.2$	$1.1 \pm 0.04$	$2.1 \pm 0.5$
1042	F	7.3	5.7	$^{238}\text{Pu}$	2.959	Bone	$6.4 \pm 1.0$	$4.2 \pm 0.2$	$4.5 \pm 0.5$	$1.9 \pm 0.2$
1027	M	7.5	5.9	$^{238}\text{Pu}$	2.148	Bone	$1.7 \pm 0.01$	<1	<1	<1
1092	M	7.0	5.4	$^{238}\text{Pu}$	1.848	Bone	$2.8 \pm 0.5$	$1.7 \pm 0.2$	$1.2 \pm 0.1$	<1
1189	M	6.6	5.0	$^{238}\text{Pu}$	0.071	N <sup>(b)</sup>	$16.0 \pm 1.6$	$9.0 \pm 0.2$	$11.0 \pm 2.4$	$1.0 \pm 0.2$
1312	M	5.7	4.1	$^{238}\text{Pu}$	0.058	N	$26.7 \pm 9.9$	$14.2 \pm 1.2$	$21.5 \pm 1.9$	$3.4 \pm 0.7$
1328	F	5.1	3.5	$^{239}\text{Pu}$	3.440	ND <sup>(c)</sup>	$8.8 \pm 0.9$	$5.0 \pm 0.1$	$1.2 \pm 0.3$	$0.9 \pm 0.1$
1371	M	5.0	3.0	$^{239}\text{Pu}$	3.060	ND	$8.7 \pm 0.7$	$2.0 \pm 0.4$	$3.4 \pm 0.2$	<1
1377	M	5.1	3.7	$^{239}\text{Pu}$	2.260	ND	$9.0 \pm 0.3$	$8.9 \pm 0.1$	$4.2 \pm 0.8$	<1
1276	F	5.7	4.1	$^{239}\text{Pu}$	2.957	ND	$3.4 \pm 1.0$	NT <sup>(d)</sup>	NT	NT
864	F	9.8	8.2	$^{239}\text{Pu}$	0.801	Lung	$3.9 \pm 0.3$	$1.8 \pm 0.5$	$2.4 \pm 0.1$	<1
880	F	8.8	7.2	$^{239}\text{Pu}$	0.840	Lung	$2.7 \pm 0.6$	$1.8 \pm 0.3$	$1.4 \pm 0.3$	<1
888	M	8.8	7.2	$^{239}\text{Pu}$	0.274	Lung	$5.1 \pm 0.6$	$3.8 \pm 0.7$	$3.4 \pm 0.2$	$1.2 \pm 0.1$
752	M	9.8	8.2	$^{239}\text{Pu}$	0.062	Bone	$2.4 \pm 0.3$	$1.5 \pm 0.02$	$1.5 \pm 0.1$	<1
701	F	10.1		C <sup>(e)</sup>			$10.7 \pm 0.8$	$9.1 \pm 0.7$	$5.4 \pm 0.4$	$1.5 \pm 0.1$

(a) Mitogen Stimulation Index =  $\frac{\text{incorporated cpm of stimulated lymphocytes}}{\text{incorporated cpm of unstimulated lymphocytes}}$

(b) N - None detected by histological examination

(c) ND - Not determined

(d) NT - Not tested

(e) C - Unexposed controls

**TABLE 69.** Mitogen Stimulation of Mesenteric Lymph Node Lymphocytes from Control and Exposed Beagle Dogs

Dog No.	Sex	Age, yr	Years After Exposure	Inhaled Plutonium Oxide	Initial Alveolar Deposition, $\mu$ Ci	Tumor	Mitogen Stimulation Index <sup>(a)</sup> $\pm$ SD			
							Con-A	PHA	PWM	LPS
1037	M	7.4	5.8	<sup>238</sup> Pu	4.854	Bone	44.5 $\pm$ 5.4	21.7 $\pm$ 3.5	30.2 $\pm$ 4.4	< 1
1042	F	7.3	5.7	<sup>238</sup> Pu	2.959	Bone	67.9 $\pm$ 7.6	44.7 $\pm$ 7.7	43.5 $\pm$ 3.7	1.1 $\pm$ 0.1
1027	M	7.5	5.9	<sup>238</sup> Pu	2.148	Bone	208.4 $\pm$ 3.4	130.3 $\pm$ 1.0	97.0 $\pm$ 5.4	< 1
1092	M	7.0	5.4	<sup>238</sup> Pu	1.848	Bone	58.5 $\pm$ 5.8	23.8 $\pm$ 8.4	21.7 $\pm$ 4.5	1.2 $\pm$ 0.2
1189	M	6.6	5.0	<sup>238</sup> Pu	0.071	N <sup>(b)</sup>	49.1 $\pm$ 6.8	24.0 $\pm$ 5.1	20.6 $\pm$ 1.5	1.2 $\pm$ 0.1
1312	M	5.7	4.1	<sup>238</sup> Pu	0.058	N	67.3 $\pm$ 12.7	35.4 $\pm$ 0.6	15.5 $\pm$ 0.3	3.1 $\pm$ 0.8
1328	F	5.1	3.5	<sup>239</sup> Pu	3.440	ND <sup>(c)</sup>	163.1 $\pm$ 18.9	57.4 $\pm$ 6.6	1.3 $\pm$ 0.3	2.2 $\pm$ 0.5
1371	M	5.0	3.0	<sup>239</sup> Pu	3.060	ND	173.9 $\pm$ 11.8	67.9 $\pm$ 3.4	68.0 $\pm$ 20.3	1.7 $\pm$ 0.2
1377	M	5.1	3.7	<sup>239</sup> Pu	2.260	ND	64.8 $\pm$ 11.5	22.6 $\pm$ 5.0	28.6 $\pm$ 2.8	1.4 $\pm$ 0.2
1276	F	5.7	4.1	<sup>239</sup> Pu	2.957	ND	33.7 $\pm$ 2.6	NT <sup>(d)</sup>	NT	NT
864	F	9.8	8.2	<sup>239</sup> Pu	0.801	Lung	77.3 $\pm$ 3.9	35.6 $\pm$ 4.4	62.5 $\pm$ 7.3	2.2 $\pm$ 0.3
880	F	8.8	7.2	<sup>239</sup> Pu	0.840	Lung	16.4 $\pm$ 1.0	11.9 $\pm$ 0.1	9.8 $\pm$ 1.1	< 1
888	M	8.8	7.2	<sup>239</sup> Pu	0.274	Lung	70.7 $\pm$ 3.2	17.1 $\pm$ 4.6	26.5 $\pm$ 3.9	1.9 $\pm$ 0.6
752	M	9.8	8.2	<sup>239</sup> Pu	0.062	Bone	94.9 $\pm$ 22.2	35.9 $\pm$ 12.9	27.1 $\pm$ 1.2	2.2 $\pm$ 0.1
701	F	10.1		C <sup>(e)</sup>			80.4 $\pm$ 12.2	33.0 $\pm$ 7.1	34.2 $\pm$ 1.7	2.1 $\pm$ 0.5

(a) Mitogen Stimulation Index =  $\frac{\text{incorporated cpm of stimulated lymphocytes}}{\text{incorporated cpm of unstimulated lymphocytes}}$

(b) N - None detected by histological examination

(c) ND - Not determined

(d) NT - Not tested

(e) C - Unexposed controls

apparent trend in the SIs of these lymphocytes with Con-A, PHA, PWM or LPS have been observed in dogs exposed to high (2 to 4  $\mu$ Ci) or low (<0.1  $\mu$ Ci) levels of plutonium oxide (<sup>238</sup>Pu or <sup>239</sup>Pu).

The reduction in stimulation indices of spleen cells correlates with the reduced responsiveness observed with peripheral-blood lymphocytes, and suggests that immunocompetence of plutonium-exposed dogs is impaired at the cellular level.