• Viral and Radiation Carcinogenesis

The studies included under this project are concerned with basic biological and biochemical indices that may aid in the detection and understanding of the primary effects of radiation insult and the initiation of the observed malignancies. A primary objective is to determine the role of virus in "radiation-induced" malignancies and in the process to identify those changes (biochemical, virological, immunological, etc.) which might serve to monitor the oncogenic process. Specific efforts include studies of leukemia induced by the β-emitter ^{90}Sr, and lung and bone tumors induced by inhalation of the α-emitters, ^{238}Pu and ^{239}Pu.

IN VITRO CELL-MEDIATED IMMUNITY ASSAY USING ^{125}I-IODODEOXYURIDINE

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We investigated an in vitro cell-mediated immunity assay using incorporation of ^{125}I-iododeoxyuridine as an indicator of lymphocyte responsiveness to mitogen stimulation. The system permits the use of whole-blood cultures in rats and dogs.

Inhaled plutonium oxide has been shown to induce a dose- and time-dependent lymphopenia prior to tumor formation in beagle dogs. In rats, a transient lymphopenia has been observed following exposure to plutonium, with peripheral blood lymphocyte numbers returning to normal levels by 1 month postexposure. The canine lymphopenia has been characterized as a decrease in peripheral blood levels of both T-(thymus-dependent) and B-(thymus-independent) lymphocytes, with greater reduction in B-cell populations. Antibody response studies indicated that plutonium exposure induced a significant functional decrease ($P > 0.01$) in primary antibody response of exposed dogs to keyhole limpet hemocyanin (Annual Report, 1977).

We are developing an assay for determining the effects of inhaled plutonium on the mitogen-induced activation of canine and rat lymphocyte populations in peripheral blood, spleen, and lymph nodes as in vitro correlates of cell-mediated immunity.

Since mitogens can generally be characterized by their degree of lymphocyte population specificity (i.e., whether they stimulate T- and/or B-lymphocytes), a series of T- and B-cell-specific mitogens were tested in the assay system. Specific mitogens tested were: Concanavalin-A and phytohemagglutinin (plant lectins that stimulate T-cell populations), bacterial lipopolysaccharide (a B-cell-activating endotoxin), dextran sulfate (a B-cell-activating polysaccharide), and pokeweed mitogen (plant lectin, mainly specific for B-cells, but also stimulates a small fraction of the T-cell population).

Lymphocyte activation was measured by the incorporation of ^{125}I-iododeoxyuridine (^{125}IUdR), a gamma-emitting thymidine analog, in newly synthesized DNA. Fluorodeoxyuridine, a known inhibitor of thymidylate synthetase, was added simultaneously with the uridine pulse to preferentially increase the DNA incorporation of ^{125}IUdR. The ^{125}IUdR is stereochemically similar to thymidine; therefore, ^{125}IUdR was incorporated into nuclear DNA in place of thymidine. The use of a gamma-emitting label (^{125}IUdR) allows us to use whole-blood cultures, thus eliminating the nonspecific stimulation of lymphocytes that results from isolation procedures.

Representative Concanavalin-A stimulation curves for dog and rat whole blood are shown in Figure 3.44. Optimal incorporation of IUdR in dog blood was observed at a concentration of 20-30 µg Concanavalin-A/ml; the response decreased at higher concentrations. In rat blood there was a much broader response: from 5-28 µg of Concanavalin-A/ml, optimally. An incubation time of 3 days was chosen as optimal for these studies.

The responsiveness of rat and dog lymph-node cell preparations is shown in Figure 3.45. Concanavalin-A concentrations required for maximal stimulation were lower in lymph-node preparations than in whole blood. In dog preparations, 3 to 5 µg/ml of Concanavalin-A was required; for rat cells, 1.5 to 3 µg. Peak responses for Concanavalin-A and the other mitogens tested are in Table 3.49.

Of the two T-cell mitogens tested, Concanavalin-A induced the higher incorporation of IUdR, probably reflecting its action on both mature and immature T-cells. Pokeweed mitogen produced higher incorporation of IUdR in rats than in dogs. The lipopolysaccharides used in these studies did not appear to stimulate rat or dog cells in either whole blood, in spleen, or in lymph-node cultures; while we have shown, in other studies, that lipopolysaccharides induce incorporation of IUdR in mouse spleen cells.

Experiments in the coming year will utilize this system to investigate the effect of inhaled plutonium on immune responses of rats and dogs.

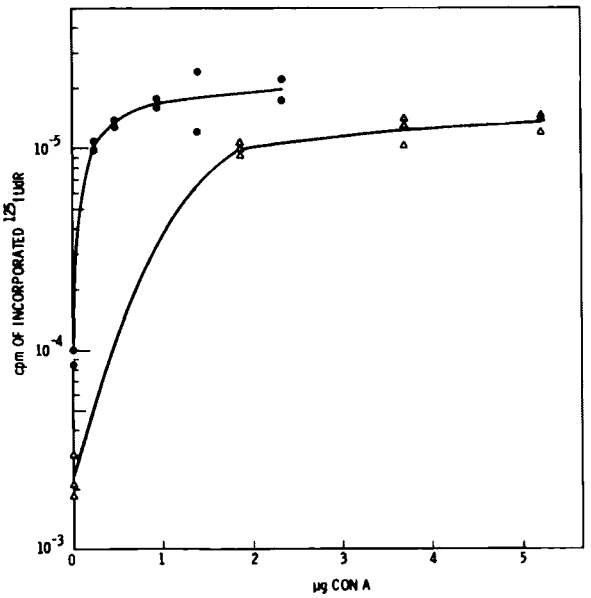

FIGURE 3.44. ^{125}I-IUdR Incorporation After 3-Day Incubation of Rat (•) and Dog (Δ) Whole Blood with Increasing Concentrations of Con-A.

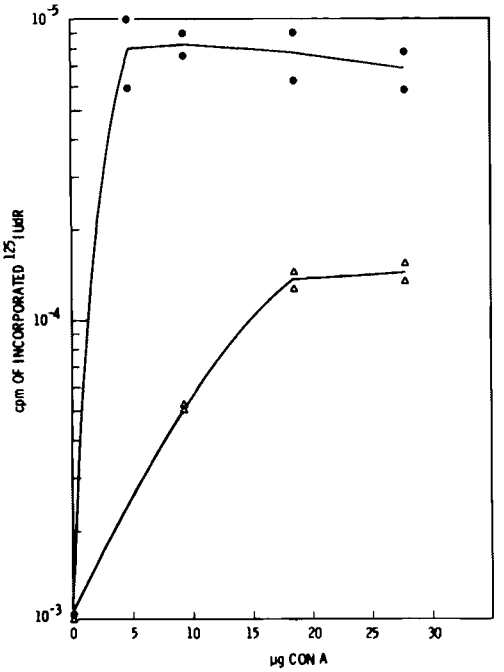

FIGURE 3.45. ^{125}I-IUdR Incorporation by Lymph Node Cells Incubated with Increasing Concentrations of Con-A (rat, •); (dog, Δ).

TABLE 3.49. Mitogen Stimulation of Lymphocytes.

	Counts per Minute x 10^{-3} of Incorporated ^{125}IUdR[a]						
	Bkg[b]	PHA[c]	ConA[d]	LPS[e]	PWM[f]	DS[g]	F[h]
Rat							
Spleen	9	153	180	NR[i]	50	NR	NR
Lymph Node	9	161	201	NR	72	NR	NR
Whole Blood	1	46	83	NR	16	NR	NR
Dog							
(Pu Exposed)							
Spleen	2	6	8	NR	6	NT[j]	NT
Lymph Node	2	56	138	NR	51	NT	NT
Whole Blood	1	NR	NR	NR	NR	NT	NT
(Unexposed)							
Whole Blood	1	NT	15.5	NR	NT	NT	NT

[a]Maximal counts incorporated after 3-day incubation with appropriate mitogen
[b]Background
[c]Phytohemagglutinin
[d]Concanavalin A
[e]Lipopolysaccharide
[f]Pokeweed mitogen
[g]Dextran sulfate
[h]Ficoll
[i]NR—no response
[j]NT—not tested