Studies on Lymphocytes. I. Lymphopenia Produced by Prolonged Extracorporeal Irradiation of Circulating Blood

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There are numerous conflicting concepts in respect to the function, fate and life span of lymphocytes. Radiation of segments of the whole-body or of lymphocytes in vitro has long been exploited in the study of lymphocytes. However, irradiation of the whole-body or segments injures lymphopoietic tissue and the in vitro environment separates the lymphocyte from homeostatic influences. We reasoned that if it were possible to destroy large numbers of circulating lymphocytes by irradiation of the circulating blood in an extracorporeal shunt under controlled conditions and without direct radiation of lymphopoietic tissues, we might be able to study many facets of lymphocyte production, life span and fate not possible or practical by other methods. This is not the initial study on extracorporeal irradiation of circulating blood, since O'Brien—with other objectives in mind—studied it in rabbits and dogs. However, we believe this is the first presentation of the feasibility of producing a blood lymphopenia in substantial numbers by extracorporeal irradiation of the blood. In this study, the blood is flowing at a constant rate under conditions such that the transit and integral radiation dose to the cells can be computed.

Methods and Materials

Animals: Preliminary studies on dogs and swine under general anesthesia demonstrated the destruction of lymphocytes, but these studies were difficult to interpret because of the marked effect of general anesthesia upon the leukocyte picture of these animals and the inability to prolong anesthesia for many hours without deleterious effect on those animals. The calf is an excellent animal for these studies because the calf can be easily restrained and the operative procedures can be carried out under local anesthesia. In figure 1 is shown a calf with cannulae in the external jugular vein, with blood being pumped through the external shunt. Calves weighing 200–400 lb. were used.

Operative procedures: Two per cent local xylocaine anesthesia was used. The external jugular vein was exposed by blunt dissection. After mobilization of the vein, one half-hour was allowed for hemostasis before the insertion of cannulae and heparinization. The Tygon cannulae, measuring OD % inch and ID % inch, are inserted into the jugular towards the head for outflow from the animal and towards the heart for the return; thus, the jugular blood flow is shunted through an external circuit. The cannulae were tied in place by #0 chromic catgut. The wound was closed with sutures for maintaining hemostasis in the skin during heparinization. At the conclusion of the extracorporeal pumping, the wound was reopened, the cannulae were removed and the vein was either ligated or sutured.

Heparinization: Six hundred mg. of heparin was given at the time the cannulae were
Fig. 1.—Experimental setup for extracorporeal irradiation. Upper left: schematic diagram of machine with extracorporeal irradiation system. Upper right: photograph of machine with extracorporeal irradiation system. Lower right: photograph of patient with extracorporeal irradiation system.
inserted and a continuous intravenous infusion of heparin was maintained at a rate of 100 mg per hour.

*The extracorporeal pump:* Details of the pump will be published in detail elsewhere. For all of these studies, the pump was operated at 70–80 strokes per minute with stroke volume 3.7–4.2 ml for a minute volume of approximately 300 ml per minute. As much of the tubing as possible was surrounded by a water jacket in order to maintain the temperature of the blood as close to the body temperature of the calf as possible. In addition, an infrared lamp was used to heat the coil.

*Irradiation:* The x-ray machine is a 250 KVP General Electric Maxitron with a 360° head. It was operated at 250 KVP and 30 m.a. The inherent filtration from the tungsten target produces an HVL of 1.75 mm Cu. The dose rate is about 300 r/min. at the midpoint of the irradiation coil around the x-ray tube. An integrating dosimeter monitored the exit dose. The integral dose has been checked by chemical dosimetry.

The irradiation device (fig. 1) is a circular frame with a diameter of 50 cm. There are roughly 25 meters of Tygon tubing coiled around the frame with an internal volume of about 900 ml. The transit time for a unit of fluid to pass through the coil is about three minutes. The transit dose is about 900 r. The integral dose to the blood cells follows a probability function based upon the blood volume, mixing of the irradiated blood in the total blood volume, transit time, dose rate, and the probability of repetitive transits through the irradiation coil. The mathematical method for this computation has been established and will be published separately.

The irradiation coil, pump, and connecting tubing were washed with running water, filled with 30 per cent H₂O₂ for at least 20 minutes to oxidize any pyrogen contaminants, washed with sterile pyrogen-free saline, and then filled with 70 per cent ethanol. Immediately before cannulation, the ethanol was pumped out and two L. of sterile pyrogen-free saline was pumped through the device to remove the ethanol. The pump, connecting tubing, and irradiation coil contained about 1200 ml of sterile saline at the time of cannulation; this is pumped into the animal after the pumping is started, thus diluting the blood by saline; the intracorporeal blood volume remains the same, as suggested by a constant hematocrit after the dilution has occurred.

**Hematologic procedures:** Microhematocrits were performed with the International centrifuge spinning at 12,000 rpm for 6½ minutes.

Platelet counts were performed by the method of Brecher and Cronkite. Leukocyte counts were performed by means of a Coulter Electronic Counter. Blood smears were made on coverslips and stained by the Wright-Giemsa method. Two hundred cell differential counts were made.

Blood samples in the initial studies were taken from the outflow tube as well as from the inflow tube to the animal after the circuit through the irradiation coil. The samples were taken at regular intervals after starting the pump and irradiation. Blood counts were performed on these samples immediately, and after six hours of incubation at 37 C., in order to allow irradiation injury to develop in the in vitro environment by the method of Trowell.

Rectal temperature was measured at half-hour intervals by a rectal thermistor, as well as the blood, coil and water sheath temperatures.

**Results**

*Leukocytes:* In figures 2 through 7, the results are plotted. In figure 3 it will be noted that a prompt lymphocytosis and neutrophilic leukocytosis develops following the heparinization, operation and during the period of pre-irradiation pumping. This has been a constant finding as in figures 3, 4 and—to a lesser degree—in figure 7. In figure 5 the influence of the combined operation, heparinization pumping and irradiation, in producing a granulocytosis is illustrated. It is of interest that the granulocytes go through a maximum while the blood is being irradiated. Apparent radiation injury of granulocytes is detected by
Fig. 2.—Effect of heparinization and two hours of extracorporeal irradiation upon the circulating lymphocyte count, and the lymphocyte count after six hours of in vitro incubation.

incubating the irradiated blood for six hours in vitro; this allows injured cells to disintegrate and results in a lower neutrophil count.

Figures 2–4 show the acute effects on the lymphocytes and figures 6 and 7 show the delayed recovery.

Following the commencement of irradiation of the blood, there is a prompt diminution in the concentration of the peripheral lymphocytes. Two hours of radiation depresses the blood lymphocyte level (fig. 2). The lymphocyte level had decreased at four hours after commencement of radiation to 55 per cent of the maximum value observed (five hours after starting the pump). Six hours of radiation produces further lymphopenia (fig. 3). The lymphocyte counts increased for two hours after heparinization and pumping, and then irradiation of the blood commenced. After six hours of irradiation of blood in the extracorporeal shunt, the lymphocyte count was 40 per cent of the peak value. The normal circulation was reestablished and the following day the lymph level was down to 33 per cent of the peak count. Twelve hours of irradiation produced a further effect (fig. 4). The lymphocytes fell to 1300 per mm.³ or 17 per cent of the level at the commencement of irradiation.
In figure 6 the slow recovery in the peripheral lymphocyte level after three hours of irradiation is shown. Lymphocytes fell from 5,400 per mm.\(^3\) at the commencement of irradiation to 3,200 at completion, or 57 per cent of the value at commencement, and continued to fall for the next 24 hours to 1600 per mm.\(^3\). Thereafter counts commenced to increase, but had not attained the pre-irradiation level even at 28 days after completion of irradiation.

In figure 7 the immediate and long-term influence of six hours of irradiation is shown. The lymphopenia is similar to previous experiments and the sluggish return is evident, still being below the pre-irradiation level 29 days after the irradiation. In this figure, the leukocytosis with quick return to normal values is illustrated.

**Platelets, hematocrit and rectal temperature:** The duration of irradiation had no effect on the platelet count. The hematocrit was reduced in proportion to the dilution of the blood volume by the amount of saline with which the extracorporeal circuit was primed. Upon completion of the extracorporeal shunt and return of all of the blood to the animal, there was a rapid adjustment of blood volume and the hematocrit returned to the usual value.

**In vitro incubation of blood:** In figures 2 and 4 the influence of six hours
Fig. 4.—Effect of heparinization and twelve hours of extracorporeal irradiation upon the circulating lymphocyte count, and the count after six hours of in vitro incubation.

of incubation of the effluent blood from the radiation coil at 37 C. is shown. Cells which have passed through the shunt only a single time and which have received about 900 rads are drastically effected, showing a dissolution of 87 per cent of the cells in a six-hour incubation period. Note in figures 2 and 4 that relatively few cells collected prior to commencement of irradiation disintegrate during six-hours of incubation. It is presumed that this latent radiation injury, not detectable by immediate cytologic observation in vitro but brought out by incubation, is "sensed" by the reticuloendothelial system promptly and that the cells or fragments are removed from circulation, thus producing the observed in vivo lymphopenia.

Discussion

It has been shown that extracorporeal irradiation of the circulating blood will damage lymphocytes. These lymphocytes will disintegrate in vitro. Prolongation of the extracorporeal irradiation produces a progressive lympho-
penia. In addition to the lymphopenia, a temporary neutrophilic leukocytosis is produced. There is no influence upon the platelet count.

The mechanism of the lymphopenia is not firmly established but is believed to be due to peripheral radiation injury with subsequent removal of the lymphocytes, presumably by the reticuloendothelial system. The sluggish return of the lymphocytes to pre-irradiation levels is in marked contrast to the reported rapid return that follows peripheral depletion by draining lymphocytes from the thoracic duct lymph in the calf. The two methods of production of lymphopenia possess significant differences that may explain the differences in recovery rates. In the extracorporeal radiation, a large number of lymphocytes are injured and presumably removed from the circulation by the reticuloendothelial system. Thus, the products of the destroyed lymphocytes may influence the capacity of the lymphopoietic organs to produce new lymphocytes. Perhaps the products of destruction of large numbers of lymphocytes over a short period of time acts as a "negative feedback" for new cell production that is similar to the inhibition of new red cell
Fig. 6.—Effect of heparinization and three hours of extracorporeal irradiation upon the lymphocyte count promptly; followed by the slow rate of recovery.

production by hypertransfusion of red cells or new platelet production by hypertransfusion of platelets. When lymphocytes are drained from the body, these cells are lost and cannot inhibit new production. Perhaps the loss from the body without feedback by products of destruction removes a brake upon new cell production. This concept is admittedly highly conjectural but for the sake of discussion one can propose the following hypothetical scheme for regulation of lymphocyte production. Normally, lymphocytes in large part presumably recycle from blood to lymph. Thus, lymphocyte production may be influenced by the presence of lymphocytes in transit through the lymph nodes. If, in the course of lymphocyte senescence, their products of destruction within nodes act as an inhibitory influence on new production, the removal of lymphocytes by external drainage should allow an accelerated production rate. If a large surplus of “inhibitory material” were to be dumped into the lymphopoietic tissues by peripheral radiation injury of cells and the subsequent phagocytosis and destruction within lymphopoietic tissues, one might obtain a depression in new lymphocyte production. On the other hand, if
Fig. 7.—Effect of heparinization and six hours of irradiation upon the lymphocyte and neutrophil count. Note prompt return to normal values with the neutrophils and the slow recovery rate of the lymphocytes.

lymphocytes are long-lived, (i.e., for as long as 100 days, indicated by the labeling studies in rats\textsuperscript{22,23}), and there is a set production rate without any mechanism for increasing this rate significantly, then one would expect a slow recovery of the lymphocyte mass. This would probably be manifested by a prolonged lymphopenia. Admittedly these are hypothetical considerations that do not answer the questions at hand. However, we believe that this method of lymphocyte depletion by extracorporeal irradiation of the circulating blood, in combination with enumeration of the outflow of lymphocytes from the lymphocytic ducts, the cytology of lymph nodes, and DNA labeling technics, will go far in obtaining direct answers to the following important questions:

1. What is the magnitude of lymphocyte recycling from blood to lymph?
2. What are the mechanisms of regulating lymphocyte production?
3. The life span and production rates of lymphocytes?
4. The stem cell capabilities of lymphocytes?
5. The products of destruction of lymphocytes?
SUMMARY

1. A method for extracorporeal irradiation of the circulating blood has been developed.
2. Extracorporeal irradiation of the blood will produce a lymphopenia promptly which persists for weeks.
3. Heparin in high doses in the calf produces a lymphocytosis and neutrophilic leukocytosis.

SUMMARIO IN INTERLINGUA

1. Esseva disveloppate un methodo pro le irradiation extracorporee de sanguine circulante.
2. Irradiation extracorporee del sanguine promptemente produce un lymphopenia que persiste durante septimanas.
3. Heparina in alte doses produce in le vitello un lymphocytose e un leuco- cytose neutrophilic.

REFERENCES


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