17052

A Plasma Factor Responsible for *in vitro* Lysis of Leucocytes by Tuberculoprotein.*

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Rich and Lewis,[1] studying living tissue cultures of washed leucocytes, showed that tuberculin in proper concentration had a selective toxic effect on cells from tuberculous animals. Since this phenomenon occurred even when allergic cells were suspended in the plasma of a normal animal, it was felt that this manifestation of tuberculin sensitivity was not dependent on circulating plasma antibodies of the sensitized animal but rather was a property inherent in the cells. Subsequent tissue culture studies[2-5] have confirmed this specific cytotoxic effect of tuberculin on leucocytes from tuberculous animals and again the reaction has been attributed to a property of the cells rather than to any serum antibody. Chase[6] and others[7,8] demonstrated that the tuberculin type of hypersensitivity could be transferred passively to normal guinea pigs by injection of the cells of peritoneal exudates, lymph nodes or spleens of guinea pigs sensitized to tuberculin by the injection of heat killed tubercle bacilli, thus confirming some crucial property of cells as the basis of delayed, tuberculin-type hypersensitivity.

More recently, it has been shown[9] that bacterial products of the tubercle bacillus exert a significant lytic effect *in vitro* on leucocytes from tuberculous animals after a one-hour period of incubation. A similar cytotoxic response has been observed on white cells from tuberculous humans.[10] Since these human white cells were suspended in normal serum (or plasma), it was concluded that the cytotoxic action of tuberculin was specific to tuberculous-type white cells and not dependent on any serum component.

It is now apparent that if such tuberculous-type white cells are thoroughly washed and then suspended in normal plasma, no lytic effect by tuberculin can be demonstrated over the one hour period of incubation. But if such washed cells, or even white cells from a normal tuberculin-negative human, are added to the plasma of a tuberculous human in the presence of old tuberculin, white cell lysis occurs during the course of one hour. It is the purpose of this report to demonstrate that a factor in tuberculous plasma is necessary for the lysis of white cells by tuberculin.

Experimental. Using a method previously described,[11] white cells from normal tuberculin-negative humans and from tuberculous patients hospitalized for acute tuberculous infection were concentrated and thoroughly washed with isotonic saline solution. The resulting cell concentrates were then suspended in normal human plasma as well as in tuberculous plasma, the amount of plasma being

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TABLE I.
In vitro Effect of Tuberculin on Blood Leucocytes in the Presence of Normal and Tuberculous Plasma.

Normal cells + Normal plasma*	0.4†	0.4						
Normal cells + Tuberculous plasma			0.4	0.4				
Tuberculous cells + Normal plasma					0.4	0.4		
Tuberculous cells + Tuberculous plasma							0.4	0.4
Tuberculin antigen	0.1		0.1		0.1		0.1	
Saline		0.1		0.1		0.1		0.1
Total WBC								
5 min.	7,760	7,440	7,810	8,070	11,500	12,150	5,070	4,000
60 min.	7,790	7,450	5,980	8,130	11,466	12,220	3,870	4,050
% decrement	+0.5	+0.3	—23.2	+0.8	—1.0	+0.5	—23.8	+1.2

* Normal cells and normal plasma are obtained from a healthy tuberculin-negative subject.
† Amounts refer to cubic centimeters.

adjusted so that final cell concentrations varied between 4,000 - 15,000 cells per cu mm. To 0.4 cc of such white cell suspensions was added 0.1 cc of tuberculin antigen (prepared as described earlier[11]). As cell system controls, 0.1 cc of isotonic saline was added to a duplicate series of cell suspensions. The test tubes used in these experiments have been coated with organosilicone to diminish nonspecific cytolysis. White blood counts were done with a mechanical pipette filler and calibrated pipettes (15 squares on 2 chambers of the standard hemocytometer slide were counted). Cytolysis was demonstrated by doing total white counts before and after a 60 minute period of incubation at 37°C.

Results. A sample protocol illustrates the results obtained in typical experiments.

1. White cells from normal tuberculin-negative humans as well as from tuberculous patients can be lysed when suspended in the plasma of tuberculous patients in the presence of old tuberculin.

2. Adequately washed tuberculous cells will show no cytolysis by tuberculin when such cells are suspended in normal plasma.

3. Such cytolysis by tuberculin varies between 20-35% of the total cells present under conditions of these experiments.

4. When isotonic saline is substituted for the tuberculin antigen, no cytolysis beyond 2.2% could be demonstrated. This variation in cell count is felt to be the amount of error inherent in the method used.

Discussion. From the foregoing, it seems apparent that tuberculin antigen can exert a cytotoxic effect on washed normal tuberculin-negative human white cells as well as on those of tuberculous patients provided tuberculous plasma (or serum) is present in the system. Results of an earlier report[10] in which tuberculous cells were similarly lysed in normal serum were probably due to the presence of traces of tuberculous plasma on the surfaces of unwashed or insufficiently washed white cells. Since it has been shown[11] that normal tuberculin-negative human white cells have the same capacity to adsorb tuberculin onto their cell surfaces as do tuberculous cells, it would appear that the vital factor in cytolysis of white cells by tuberculin is some component in tuberculous plasma. Further experiments to elucidate the nature and origin of this plasma factor are now in progress.

Summary. Under the conditions of the experiments reported here, the cytolysis of human white blood cells by tuberculin occurs

only in the presence of plasma from tuberculous subjects. White blood cells from healthy tuberculin-negative humans will undergo similar tuberculin cytolysis in the presence of such tuberculous plasma.

17053

The Role of the Spleen in Radiation Injury.*

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Ectopic blood formation in the spleens of mice injected with a dose of 2.0 microcuries per gram of body weight of radiostrontium (Sr^{89}), as shown by Jacobson *et al.*,[1,2] was sufficient to obviate the development of anemia even though the bone marrow was largely destroyed and only gradually reconstituted over a period in excess of 100 days. Splenectomized mice given this dose developed a severe anemia, recovery from which occurred only as the hematopoietic activity of the bone marrow recovered. This communication describes a somewhat different but related technic for studying the significance of the spleen in recovery from or compensation for radiation injury.

Materials and Methods. Four groups of young female mice were prepared as indicated in Table I. Mice in Group I were untreated controls. The mice in Groups II, III, and IV were anesthetized, an incision made in the left upper quadrant of the abdomen, and the spleen brought out through the abdominal incision with the main pedicle intact. Group III and Group IV mice were irradiated with 600 r whole-body X radiation (250 Kv) except that during the irradiation the mobilized spleens of Group IV mice were placed in one-quarter inch thick lead boxes with openings for the pedicle only. The mobilized spleens of Group III mice were placed in thin paraffin boxes which offered no appreciable shielding from the radiation. The mobilized spleens of Group II mice (operated controls) were placed in lead boxes for a period equal to the time that Group III and Group IV spleens were thus contained. The radiation required approximately 12 minutes after which the spleens of groups II, III, and IV mice were returned to the abdominal cavity and the operative incisions sutured.

Results. Hematologic studies were made on all 4 groups. Animals from each group were sacrificed at intervals for histopathologic study.

The mean hemoglobin, erythrocyte, and hematocrit values of Group IV (lead-protected spleens) were not significantly altered when compared to control Groups I and II (Fig.

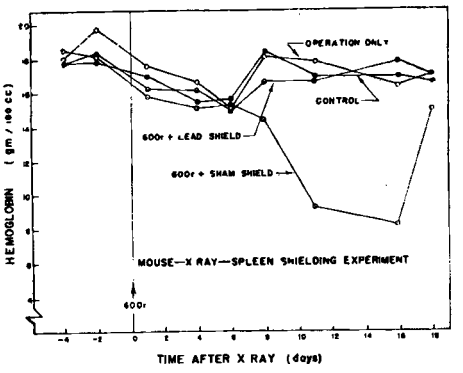

FIG. 1.
The hemoglobin values of control mice and mice exposed to 600 r with and without lead protection of the spleen.

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