SITE OF FORMATION OF LEUKOPOIETINS IN DOGS WITH ASEPTIC INFLAMMATION

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Blood was taken from the femoral artery, femoral vein, veins of the spleen, liver, kidney, stomach, and intestine and from the right heart of dogs on the 3rd and 7th days of aseptic inflammation (at the time of maximal accumulation of leukopoietins in the blood) and the leukopoietic activity of the serum was studied. Inflammation was produced by subcutaneous injection of turpentine. The leukopoietic activity of the venous blood flowing from the various organs was compared with that of arterial blood. Blood flowing from the kidneys had the highest leukopoietic activity. Blood from the veins of the spleen, liver, stomach, and intestines had a lower content of leukopoietins than arterial blood.

Endogenous substances capable of inducing leukocytosis or of stimulating leukopoiesis have been given many names depending on the conditions and methods used for their detection [3]. Opinions differ regarding the site of formation of these substances.

According to Japanese workers, the factor inducing neutrophilic leukocytosis, the presence of which they demonstrated in the blood of animals after injection of typhoid vaccine, nucleic acids, and various other substances, is formed in the liver [7, 13, 18]. The same view is held, on the basis of their own investigations, by Beer [8] and Linke and Schricker [14].

Ludwig et al. [15] consider that a factor inducing leukocytosis and found in the blood after repeated leukopheresis, is formed in the spleen. It has also been suggested that a factor stimulating the release of granulocytes from the bone marrow into the circulating blood [11], and also a factor inactivating leukopoietins [6, 19, 21] or delaying their formation [14], are formed in the spleen.

Studies of the granulocytopoietic activity of extracts of various organs of intact animals have shown that kidney tissue extract has the highest activity [9, 10]. These workers concluded that the chief source of granulocytopoietin found in the blood is the kidneys. Delmonte et al. [10] do not rule out the possibility that leukopietins may be stored in the kidneys. Since the content of leukopoietins in the blood was less than in the kidney tissues, they also accept that leukopoietins may be inactivated in the blood by an inhibitor.

Menkin [16] considers that factors inducing leukocytosis and found in the blood and exudate during inflammatory conditions are formed in the inflammatory focus from damaged leukocytes.

The results of the writers' earlier investigations [4] conducted on dogs and horses with aseptic inflammation induced by turpentine demonstrated an increased blood level of leukopoietins capable of stimulating granulocytopoiesis in intact animals. The greatest accumulation of leukopoietins in the blood of dogs was observed 3 and 7 days after injection of turpentine.

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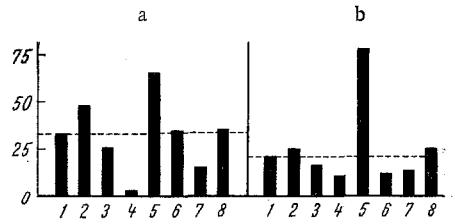

Fig. 1. Serum leukopoietic activity of blood taken from various vessels of dogs on 3rd (a) and 7th (b) days of aseptic inflammation. Abscissa: 1) femoral artery; 2) femoral vein; 3) splenic vein; 4) hepatic vein; 5) renal vein; 6) gastric vein; 7) intestinal vein; 8) right heart; ordinate, leukopoietic activity (in units).

To discover from which organs the leukopoietins enter the blood stream, in the investigation described below the leukopoietic activity was studied in serum obtained from the blood flowing from various organs of dogs with aseptic inflammation.

EXPERIMENTAL METHOD

Experiments were carried out on 12 dogs (males and females) weighing 12-22 kg. Aseptic inflammation was induced by a single subcutaneous injection of 50% turpentine (diluted with mineral oil) in a dose of 2.5 ml/10 kg body weight. Six dogs were investigated 3 days, and another six dogs 7 days after the injection of turpentine. Under morphine and thiopental anesthesia the femoral artery and vein of the animals were dissected, the abdomen was then opened, and blood samples were taken from the veins of the kidney, liver, spleen, stomach, and small intestine, and from the right heart (through a catheter inserted into the jugular vein), the femoral artery, and the femoral vein. The serum leukopoietic activity was tested on Wistar rats. The leukopoietic activity was judged from changes in granulocytopoiesis 3 days after a single intravenous injection of 1 ml of the test serum [5]. An increase of 1% in the number of immature granulocytes in the bone marrow compared with the initial value was taken as the unit of activity.

Each sample of serum was injected into 3 or 4 animals and the mean results calculated and subjected to statistical analysis. The leukopoietic activity of blood serum from the vessels specified above was compared with the activity of arterial blood. The activity of 95 samples of serum from 319 rats was studied.

EXPERIMENTAL RESULTS

The mean values for the serum leukopoietin content in blood taken from the vessels specified are given in Fig. 1. On the 3rd day after injection of turpentine the mean content of leukopoietins in a sample of serum taken from an artery was 38 units. In blood from the femoral vein and kidney the content of leukopoietins was higher than in arterial blood, but a significant increase was found only in blood from the renal vein (65 units, $P < 0.01$). The content of leukopoietins in blood from the splenic and hepatic veins was significantly reduced (25 and 3 units; $P < 0.05$ and 0.01 respectively). The leukopoietic activity in blood from the intestinal veins was lower than that of arterial blood, but the difference was not statistically significant.

Similar changes were observed after 7 days but the difference from the arterial blood was significant only in the case of blood obtained from the renal vein, in which the leukopoietin content was 4 times higher (78 units, $P < 0.01$) than in arterial blood (21 units).

The results show that leukopoietins enter the blood stream from the kidneys. These findings, indicating a role of the kidneys in leukopoietin formation, are in agreement with others published in the literature [9, 10]. The workers cited found a high content of leukopoietic activity in the kidney tissuew of intact animals. The active substance was thermostable and nonprotein in nature [10]. In inflammation 2 leukopoietins were found to be present: one thermostable, the other thermolabile [16]. The possibility cannot be ruled out that thermostable leukopoietin is formed from disintegrated leukocytes in the focus of inflammation. The leukopoietic activity of breakdown products of leukocytes has been demonstrated repeatedly [1, 2, 12, 17, 20].

After passage through the liver and spleen, the leukopoietic activity of the serum is reduced, possibly because of storage of the leukopoietins in these organs. The lowered activity of the blood flowing from the stomach and intestine is probably due to excretion of leukopoietins via the gastro-intestinal tract.

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