Cx-REACTIVE PROTEIN IN RADIATION SICKNESS

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The processes of destruction of cells and tissues occupy one of the central positions in the pathogenesis of radiation sickness [1,3], and an all-sided study of them represents significant theoretical and practical interest for radiobiology.

Together with the histological and cytological methods, which are widely used for the study of radiation sickness, the study of C- and Cx-reactive proteins has much interest for the analysis of the dynamics of tissue and cellular disintegration.

C- and Cx-reactive proteins, as is known, are specific proteins which appear in blood in the acute phase of some inflammatory and necrotic processes [2,6,7].

The high sensitivity of the reaction to C- and Cx proteins, the simplicity of its performance, and the fact that in a number of cases it is a more delicate indicator of the gravity of the pathological state than the change in the leucocyte count and the ESR, makes the performance of this reaction very expedient in the study of radiation sickness.

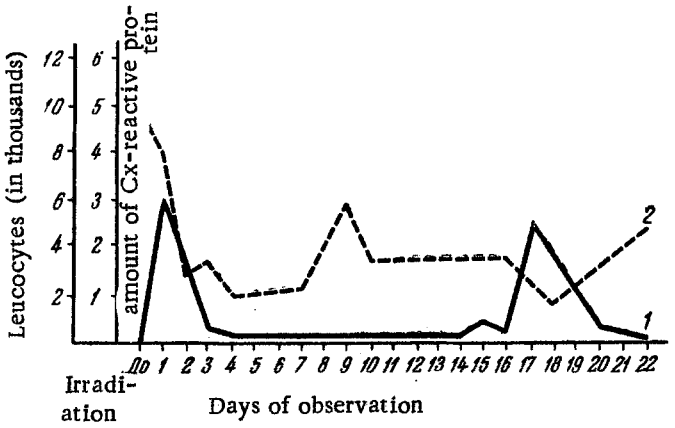

Fig. 1. Dynamics of change in amount of Cx-reactive protein and of the number of leucocytes in acute radiation sickness (800 r. irradiation). The rabbit was sacrificed on the 22nd day after the beginning of the experiment. 1) Cx-reactive protein (in millimeters of precipitate); 2) leucocytes in one mm^3 blood (in thousands).

In addition it ought to be noted that in the comparatively voluminous literature appearing in recent years on the clinical importance of the C- reactive protein in various diseases, a limited amount of work has been devoted to the dynamics of its change in radiation sickness [7].

As is known, C-reactive protein appears only in the blood of man and some species of monkeys. A protein appears in rabbits, in the acute phase of inflammatory-necrotic processes, which is closely related, immunologically, to the C-reactive protein—the so-called Cx-reactive protein.

In connection with what has been stated above, we decided it would be expeditious to study the possibility of the appearance and the dynamics of change in Cx-reactive protein during acute radiation sickness.

METHOD

The most sensitive and convenient of presently existing methods for determining C- and Cx-reactive protein is the method of capillary precipitation of the corresponding specific C- and Cx-reactive antiserum [9].

In view of our lack of commercial antisera we prepared (with the assistance of A. L. Yampol'skii) a few batches of anti C- and anti Cx-reactive sera, which had high precipitating power. Purified Cx-reactive protein was prepared by a slight variation of MacCarty's method [11]. Immunization of guinea pigs with a purified Cx-antigen was carried

out using a modification of Freud's method.

Radiation sickness in rabbits was brought about by total irradiation of the animals with x-rays, applying a dose of 800 and 1200 r.

We checked the dynamic development of the radiation sickness on the basis of the general condition of the animals and the number of leucocytes in the peripheral blood. We carried out the reaction to the Cx-reactive protein at the same time as the blood analysis. The intensity of the reaction was determined by the amount of precipitated complex.

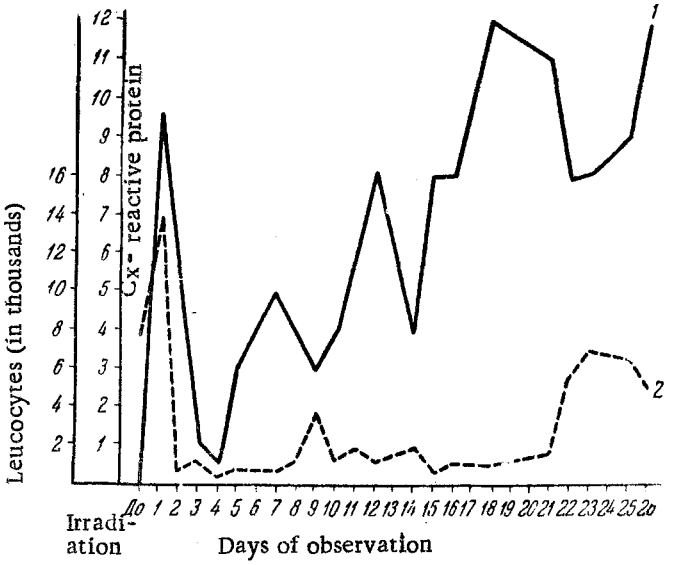

Fig. 2. Dynamics of change in the amount of Cx-reactive protein [1] and of the number of leucocytes [2] in acute radiation sickness (1200 r. irradiation). The animal died on the 26th day after the beginning of the experiment. Ordinate Axis: Left-number of leucocytes in one mm^3 blood (in thousands); right-amount of Cx-reactive protein (in millimeters of precipitate); Abscissa Axis: Days of observation; 1) Cx-reactive protein (in millimeters of precipitate); 2) Leucocytes.

RESULTS

The conclusion can be drawn, from an analysis of the experimental data we obtained, that a definite parallelism exists between the course of radiation sickness and the intensity of the Cx-reactive protein reaction.

In acute radiation sickness, induced by irradiation of rabbits with sublethal doses of x-rays (800 r), the blood of the animals gave a positive reaction for Cx-reactive protein within 18-20 hours after the irradiation. (Fig. 1) In the days following, the content of this protein in the blood gradually decreased and on the third day generally dropped to zero. The subsequent course of the radiation sickness in this event, as a rule, was not accompanied by the appearance of Cx-reactive protein, or was characterized by the periodic appearance of a weakly positive reaction.

In the case of the acute radiation sickness induced by irradiation with lethal doses (1200 r), Cx-reactive protein was also detected in the blood of animals within 18-24 hours after irradiation, during which its level, as a rule, was 2-3 times higher than in the 800 r irradiation doses. In the following 2-3 days the concentration of this protein dropped sharply and, on the 4-5th day its quantity in the blood was very small. But, whereas a negative or weakly positive reaction for Cx-reactive protein was observed during the entire latter period of the sickness induced by irradiation with sublethal doses, in the radiation sickness induced after 1200 r irradiation doses, on the other hand, a marked quantity of Cx-reactive protein once again appeared in the blood on the 5-7th day, and its content progressively increased on a level with the development of the pathological process.

On the 10-12th day, the quantity of precipitate of Cx-reactive protein reached 13 mm, i.e., the maximum for the precipitating power of the antisera (Fig. 2).

It must be pointed out that in those cases where the animal survived, the quantity of tested protein, did not, as a rule, exceed 5-7 mm in the period corresponding to the second maximum, and after the 18-20th day it decreased to zero.

Thus, it can be concluded that the dynamics of change in the content of Cx-reactive protein exactly reflects the course and gravity of the radiation sickness and in some cases it may play a role as a factor in the prognosis.

The periods of appearance of the Cx-reactive proteins in the blood of irradiated animals coincide in phase with the periods of expressed development of the cytolytic and destructive processes, induced as a result of the irradiation. The first maximum coincides in phase with the development of the expressed lymphopenia. As for the second rise in the curve which characterizes the dynamics of change in the Cx-reactive protein, it corresponds to that period of the radiation sickness when the most expressed destructive changes take place in the tissues.

This fact confirms the thinking of a number of authors [10,12] who are investigating the appearance of C- and Cx-reactive protein as the result of tissue and cellular destruction.

During our study of the dynamics of change in Cx-reactive protein during radiation sickness, we decided it would be expedient to compare the data obtained by us with the change in leucocytosis and with the content of the α-globulin fractions of serum protein, which are also extremely precise indices of the gravity of the given pathological process.

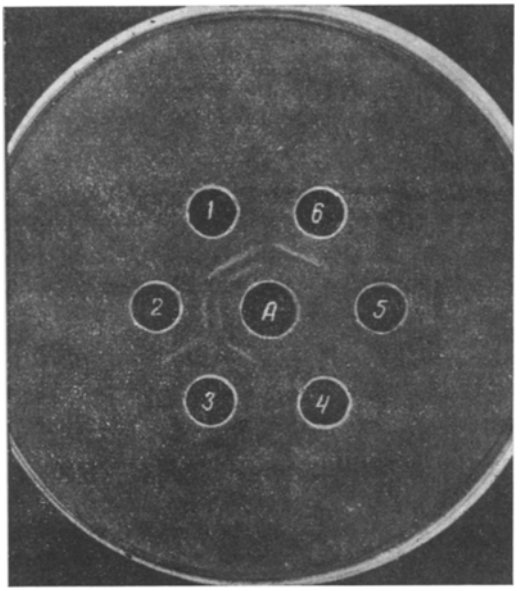

Fig. 3. Immunodiffusion test of specific antigens of the blood serum of irradiated rabbits. In the center: 4) serum from an irradiated rabbit, absorbed with antiserum for Cx-reactive protein. 1) Antiserum in serum from an irradiated rabbit (non-absorbed), two zones of precipitation visible; 2) The same as in 1, dilution 1:1, two zones of precipitation visible; 3) Antiserum in the serum from an irradiated rabbit absorbed with normal rabbit serum in proportion 1:2, one zone of precipitation is observed; 4) Physiological solution (control for the absence of non-specific precipitation); 5) Antiserum for Cx-reactive protein (the reaction is negative, protein totally absorbed); 6) Antiserum in serum from a normal rabbit (one zone of precipitation, common with serum of an irradiated rabbit).

A comparison of the curves of change in the leucocyte count and the Cx-reactive protein allows the conclusion to be drawn that parallelism in the progress of the curves indicated is the exception, rather than the rule.

In the first phase of acute radiation sickness, an increase of Cx-reactive protein may accompany both an increase, and a decrease in the leucocyte count. The second rise in the quantity of Cx-reactive protein takes place during an expressed leucopenia, and, moreover, the changes, observed in the leucocyte count, rarely correlate with the change in the quantity of this protein.

As is known, the change in the composition of the α-globulin fractions of serum protein is one of the most expressed displacements observed in the blood protein picture during radiation sickness. [4,5] Moreover, especial attention is to be paid to the appearance in the blood of anomalous α_3-globulins and their relation to Cx-reactive protein. As we reported earlier, [5] anomalous globulins appear on the 6-7th day after irradiation, and are observed for a long time in the blood circulation of the irradiated animal. Thus, the phase of maximal entry of anomalous α_3-globulins into the blood, is begun during the period corresponding to the minimal level of Cx-reactive protein in the blood.

Further, in animals afflicted with acute radiation sickness, an increase in the level of Cx-reactive protein and α_3-globulins occurs at the same time.

The very fact that both of the above-noted proteins appear in the blood at a different time is testimony that we are dealing with two different proteins, but a conclusive judgment on this question can only by given by their immunological analysis.

For this purpose, we set up a series of tests, in which, with the aid of an immunodiffusion method, it was shown that serum from irradiated animals, containing both Cx-reactive protein and α_3-globulins, after absorption with its anti Cx-reactive serum, ceases to give a reaction for Cx-reactive protein, but continues to react with an antiserum obtained in response to the administration of serum from irradiated animals (Fig. 3).

Thus, the data we obtained permits the conclusion that at least two protein components appear in blood serum during radiation sickness, which are not present in the blood of non-irradiated animals.

The problem of the pathological significance of these proteins and, also, an explanation of the mechanism for their appearance in the blood, represent the object of further research by us.

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