THE CONCENTRATION OF Y ANTIGEN IN NORMAL AND LEUKEMIC TISSUES OF MAN AND ANIMALS

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We have previously shown [3] that antisera obtained by immunization of rabbits with saline extracts of human leukemic spleen can be used to detect a hitherto unknown, heterogenous Y antigen in the tissues of mice, rats, and guinea pigs. It is found mainly in organs and tissues rich in reticulo-endothelium, and is evidently associated with certain corpuscular particles, so that it may be separated from a mixture of heterogenous antigens by means of differential ultracentrifugation. Y antigen was detected by 8 antisera (of 13 tested) against leukemic human spleen, and by one antiserum (of 29 tested) against normal, carcinomatous, and sarcomatous human tissues.

On the assumption that Y antigen plays a definite role in the pathogenesis of leukemia, we have compared the concentration of this antigen in leukemia and the corresponding normal tissues of man and animals.

EXPERIMENTAL METHOD

Antigens for the precipitation reaction in jelly were prepared by grinding tissues in a mortar with quartz and (1 g tissue and 3 ml of physiological saline). After centrifugation at 4000 rpm for 10 min, the supernatant fluid was used as antigen. Mouse and rat antigens were obtained from a mixture of liver and spleen, and human antigens from each organ separately. Rabbits were immunized with saline extracts of the spleens of persons dying from acute or subacute leukemia [3]. The antigens were studied in the precipitation reaction in jelly, using our own micromodification. In some cases Bjerklund's specific delay of precipitation reaction was used. The protein concentration in the antigens was determined by the micro-Kjeldahl method.

EXPERIMENTAL RESULTS

Y antigen is neither a Forssman nor a serum heterogenous antigen [3]. At the same time, exhaustion of antileukemic sera with saline extracts of the spleen of a healthy person suppressed the formation of precipitation lines corresponding to Y antigen. Assuming that in man, as in mice, Y antigen is present in tissues rich in reticulo-endothelium, we exhausted an antileukemic serum with antigen prepared from the lungs of a person dying from accidental injury. The exhaustion was carried out by Bjerklund's method in agar. The antileukemic serum was poured into the central well, mouse antigen into one of the peripheral wells, and antigens prepared from various human organs were poured into the others.

After complete neutralization of the antibodies to normal lung antigen, the antileukemic serum continued to react with Y antigen contained in the mouse organs. Meanwhile this antiserum caused the formation of precipitation lines against the wells containing antigens prepared from the human liver, spleen, and kidneys. The precipitation line situated against the wells with the human antigens was not fully identical with the line due to Y antigen of the mouse tissues, as shown by the formation of an additional spur. The latter indicates that the Y antigen in human tissues forms part of an antigenic complex, which also includes an antigenic component not present in mouse tissues.

Y antigen was detected in all samples of human liver, spleen, and kidneys (antigen systems were prepared from organs from 7 cadavers) and in 3 (of 7 tested) samples of lungs, but none was found in the heart, stomach, and thyroid.

Why should the Y antigen contained in the spleen of the patients with leukemia possess a much greater immunogenic activity than the same antigen in normal human tissues? We made a comparative study (using the serial

dilution method) of the concentration of Y antigen in normal and leukemic tissues from human subjects, mice, and, lastly, cattle. Antiserum obtained by immunization of rabbits with saline extracts of human leukemic spleen was poured into the central well, and serial dilutions of antigens were poured into the peripheral wells.

We give only the highest dilution at which Y antigen could still be detected (in all previous dilutions the reaction was always positive).

In the first place we studied antigens prepared from the spleens of a healthy person and a patient with leukemia. In the spleens of persons dying from accidental injury or cardiovascular diseases, Y antigen was found in the initial

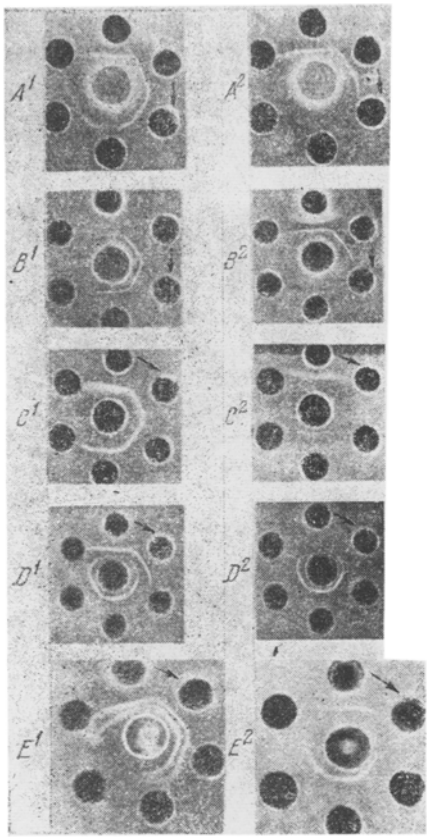
Reaction between Serum against Human Leukemia Spleen and Y Antigen Contained in Various Dilutions of Antigens

Source of test material	Organ from which antigen was prepared	No. of antigens studied	Dilution of antigens				
			initial	1:2	1:4	1:8	1:16
Person dying from aplastic anemia	Spleen	4	+	+	+	+	
with a leukemoid reaction		5	+	+	+	+	+
Persons dying from trauma or	"	5	+	—	—	—	—
cardiovascular diseases		5	+	+	—	—	—
Child dying from reticulosis	"	1	+	—	—	—	—
Person dying from aplastic anemia	"	1	+	+	—	—	—
with a leukemoid reaction							
Human embryos	"	4	—	—	—	—	—
	Liver	1	+	+	—	—	—
		1	+	+	+	—	—
Afb mice with spontaneous	Mixture of liver	3	+	+	+	+	—
leukemia	and spleen	2	+	+	+	—	—
Healthy Afb mice	The same	4	—	—	—	—	—
		1	+	—	—	—	—
C_3H (f) mice with leukemia	" "	1	+	+	+	+	+
"induced" by human leukemic							
material							
C_3H (f) mice with leukemia	" "	1	+	+	+	+	
"induced" by an uninfected		2	+	+	+	+	
culture of monkey's kidney							
Healthy C_3H (f) mice	" "	5	+	—	—	—	—
C 57 Bl mice with transplanted	" "	6	+	—	—	—	—
leukemias (Lel, La, Lb)							
Healthy mongrel albino rats	" "	1	+	+	—	—	—
		1	+	—	—	—	—
Mongrel albino rats with	" "	2	+	—	—	—	—
transplanted leukemia							
Leukemic cow	Spleen	1	+	+	+	+	+
Healthy cows	"	1	+	+	—	—	—
		1	+	—	—	—	—

Note: + Y antigen detected by antileukemic serum; — Y antigen not detected by this serum.

dilution or in a dilution of 1 : 2 (see table). In the spleens of patients dying from acute or subacute hemocytoblastosis Y antigen was found in dilutions of 1 : 8 or 1 : 16 (see figure). It was found in the initial dilution in one child aged 18 months with subacute leukemia (reticulosis) whose spleen was tested, and in a dilution of 1 : 2 in the spleen of a woman dying from aplastic anemia with a leukemoid reaction.

Y antigen either could not be detected at all in the tissues of healthy Afb mice, or it was found in the initial dilution, whereas in the Afb mice with spontaneous leukemia it was detected in dilutions of 1 : 4 or 1 : 8. In the tissues of healthy C_3H (f) mice it was found only in the initial dilution; in the C_3H (f) mice with leukemia developing after injection of leukemic human brain, with preliminary blind passages through guinea pigs, rats, and mice—in a dilution of 1 : 16 [1]; in C_3H (f) mice with leukemias induced by an uninfected culture of monkey's kidney—in dilutions of 1 : 8 or 1 : 16.



Titration of antigens A^1, A^2, B^1, B^2, C^1, C^2, D^1, D^2, E^1, and E^2 in the central wells of a serum against human leukemic spleen; in the peripheral wells—serial dilutions of antigens—1, 1 : 2, 1 : 4, 1 : 8, and 1 : 16 (in experiments with antigens from mice and cows) and 1 : 32 (in the direction of the arrow). A^1, A^2, B^1, B^2, —excess of antigen prepared from healthy human lung was first added to the agar; A^1) in the upper well—antigen from a cow's spleen, in the remaining wells—antigen from human leukemic spleen; A^2) in the upper well—antigen from a cow's spleen, in the rest—serial dilutions of antigen from a healthy human spleen; B^1) in the upper well—antigen from a mouse's spleen, in the rest—serial dilutions of antigen from human leukemic spleen; B^2) in the upper well—antigen from a mouse's spleen, in the rest—serial dilutions of antigen from normal human spleen; C^1) serial dilutions of antigen from the spleen of a leukemic cow; C^2) serial dilutions of antigen from the spleen of a healthy cow; D^1) serial dilutions of antigen from the spleen of a mouse with spontaneous leukemia; D^2) serial dilutions of antigen from the spleen of a healthy mouse; E^1) serial dilutions of antigen from a C_3H (f) mouse with leukemia "induced" by human leukemic material; E^2) serial dilutions of antigen from a healthy C_3H(f) mouse.

In contrast to the Y antigen, other heterogenous antigens such as S antigen [3] were detected in equal dilutions in the tissues of healthy and leukemic mice. The precipitation line corresponding to these antigens was situated nearer to the well with antiserum. We also studied antigens prepared from the spleens of cows and other organs of different animals. These studies showed that Y antigen was present not only in the organs of mice, rats, and guinea pigs, as was already known [3], but also in the organs of pigs, dogs, lemmings, and monkeys (Macacus rhesus). It could not be detected in the organs of hamsters and chicks. Whereas Y antigen was detected in the spleen of healthy cows in a dilution of 1 : 2, it was found in the spleen of a cow with lymphatic leukemia in a dilution of 1 : 16. The line due to Y antigen contained in the spleen of the cow was completely identical with the line formed opposite the well with purified Y antigen from mouse tissues.

Hence the concentration of Y antigen was 4-16 times higher in the spleens of patients with acute leukemia, the spleen of a leukemic cow, the tissues of Afb mice with spontaneous leukemia and the tissues of C_3H (f) mice with "induced" leukemias than in the corresponding tissues of human subjects or animals with no visible sings of leukemia.

We may postulate on the basis of these findings that this antigen is associated with young proliferating cells. Experiments were carried out with transplantable leukemias of mice and rats and with human embryonic tissues. The concentration of Y antigen in the tissues of mice with transplanted leukemias Lel (strain Gorer), La (strain Puyman) and Lbl (strain Prigozhina) and in the tissues of rats with transplanted leukemias L_{37} and ILK was no higher than in the corresponding tissues of healthy animals: in all animals it was detected only in the initial dilution.

The experiments on human embryonic tissues were of special interest. The embryos were obtained from a maternity home after artificial or spontaneous abortion. Altogether four human embryos, aged 4-8 months, were used. Antigens were prepared by the usual method from the spleen, liver, kidneys, heart, and lungs of the embryos. An excess of antigen prepared from the lungs of a person dying from accidental injury was first added to the agar. Irrespective of the age of the embryo, Y antigen was found only in the embryonic liver, and in dilutions of 1 : 2 or 1 : 4. It could not be detected in any other organs.

In face of these findings, it is doubtful if Y antigen is characteristic of all proliferative processes. In fact, the tissues of mice and rats with transplanted leukemias contained a larger number of proliferating cells, although the concentration of Y antigen detected in these tissues was no higher than in the corresponding normal tissues. The embryonic tissues also were rich in young cells, and they constitute the greater part of the mass of all organs in 4-5-month embryos. However, Y antigen was found in the human embryo only in the liver, and in a concentration only $^1/_4$-$^1/_8$ as high as in the spleen or liver of patients dying from acute or subacute hemocytoblastosis. It is possible, therefore, that the increased production of this antigen is dependent on specific biological changes characteristic of human hemocytoblastoses and of certain spontaneous leukemias of animals.

We cannot give an answer at this stage to the question why Y antigen can be detected in the human embryo only in the liver, whereas in adult human subjects it is also present in the spleen, the kidneys, and sometimes the lungs. It may be that it is present in extremely small amounts in these organs in the embryo, and could not therefore be detected in the precipitation reaction in jelly.

SUMMARY

A comparative study was made of the heterogenous Y antigen in the leukemic and normal tissues of man and animals (Y antigen is mainly determined in the organs rich in reticulo-endothelium) by means of sera against the leukemic human spleen; the antigen, bound to some corpuscular particles, has made it possible to isolate it from a mixture of other heterogenous antigens (3). The content of antigen Y proved to be 4-16 times greater in the tissues of man suffering from acute and subacute hemocytoblastoses and in the tissues of the Afb line mice with spontaneous leukemias than in the corresponding normal tissues. The content of this antigen is not greater in the tissues of mice and rats with prolonged transplantable leukemias than in those of the unaffected animals. Of all the organs of the human 4-8 month-old embryo the liver was the only organ which contained the Y antigen. The content of this antigen in the embryonic liver was not great as compared with the tissues of persons who died of trauma or of cardiovascular diseases. It is assumed that the rise of the Y antigen content is not connected with any kind of proliferative processes, but it depends upon peculiar biological changes of significance in the pathogenesis of human hemocytoblastoses and some spontaneous animal leukemias.

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