Cytotoxic Effect of a Xenogeneic Antiserum on Bone Marrow Cells From Normal or Sublethally Irradiated Mice

A population of small, mononucleated cells were shown to accumulate in the bone marrow of mice recovering from sublethal irradiation[1-4]. Ultrastructural studies indicated that this population is heterogeneous and contains, in addition to lymphocytes and lymphoblasts, small granulocytic cells and a considerable amount of particular lymphoid cell elements denoted X cells. The latter cell type has an immature character, is not detected in unirradiated normal adult marrow, but is present in the marrow of new-born mice[5]. In view of these properties, and observations on resurgence of cells with embryonic antigens in the adult organism under certain conditions[6,7], the question arose whether X cells may also represent an embryonic cell type. This article reports the results of experiments which were performed to test this possibility.

A rabbit of approximately 3 kg weight received i.v. injections of 0.5 — 1×10^9 embryonic mouse cells in 10 ml balanced salt solution (BSS) 3 times at 14 day intervals. The cells were freshly prepared from CBA embryos 15–18 days after conception by pressing the tissues through a stainless steel net, and suspending the cells in BSS. The rabbit was bled 1 week after the last injection. After separation, the serum was stored at −20 °C.

Aliquots of 1 ml of the serum were absorbed at 37 °C for 1 h, successively with red blood cells, liver cells, lymph nodes mixed with thymus, spleen and bone marrow prepared from 10–30 adult CBA mice. The ratio serum to packed cells was about 1:1 in the case of bone marrow cells and 1:3 in the case of the other cell material. 2–4 sets of absorptions were carried out.

Bone marrow cell suspensions were prepared in BSS from the femurs of 10–11-week-old CBA female mice which were either unirradiated or irradiated on the whole body with 400 R X-rays 12 days earlier (200 kV, 15 mA, HVL 0.95 mm Cu, F.D. 50 cm).

For cytotoxicity tests 50 µl aliquots of the suspension with a concentration of 10^7 cells/ml were incubated with 50 µl amounts of serially diluted serum at 37 °C for 1 h. Controls were prepared without serum. Guinea-pig serum in amounts of 50 µl and diluted 1/3 was added as complement. All dilutions were made up in BSS. The cells were kept in an ice bath during counting. Trypan blue solution (4‰) in amounts of 50 µl was added immediately before reading. The percentage of unstained cells, indicating the surviving fraction, was calculated by counting 300–500 cells in each preparation.

Table I indicates the percentage of viable cells when cells from normal (NM cells) or preirradiated marrow (XM cells) were exposed to the rabbit antiserum in different dilutions in the presence of complement. Practically all NM cells were killed by unabsorbed serum in dilutions up to 1/500. When the serum was absorbed twice with normal adult tissues, its toxicity was reduced and NM cells were not killed in dilutions above 1/32. In contrast, the twice absorbed serum showed a toxicity for XM cells in a dilution of 1/500. Two repeated experiments performed with the serum after 4 absorptions with normal adult tissue gave similar results indicating that XM cells were killed at higher dilutions and to a larger extent than were NM cells. XM cells are thus clearly more sensitive to the antibodies present in the absorbed serum than are NM cells. The difference can be attributed to a difference in the reaction of the cells to the same antibodies. It is also conceivable that the serum contains, in addition to antibodies active to both NM and XM cells, antibodies which have a specific toxicity for XM cells.

This latter possibility was tested in experiments in which cells were exposed to serum absorbed with NM or XM cells. In Table II, results from 3 experiments are shown which demonstrate that absorption of the serum with NM cells removed completely the toxicity for NM cells but not for XM cells since 20% of these were still killed at dilutions up to 1/16. For these dilutions, cytotoxic indices larger than 0.2 can be calculated. Absorption with XM cells removed the toxicity almost completely for both NM and XM cells.

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Table I. Percentage of viable cells in CBA bone marrow cell populations as determined by the trypan-blue test after incubation with antiserum produced in rabbit against CBA embryonic cells

No. of absorptions	Type of marrow	Percentage of viable cells									
		No serum	Serum dilutions								
			1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
0	NM	81	<5	<5	<5	<5	<5	<5	<5	<5	15	65
2	NM	82	<5	<5	15	40	45	80	75	75	85	—
	XM	79	<5	<5	<5	<5	<5	15	30	53	70	82
4	NM	83	66	55	70	78	83	79	—	—	—	—
	XM	79	16	19	35	49	66	52	74	75	—	—
4	NM	84	70	69	80	76	—	—	—	—	—	—
	XM	77	35	23	29	37	43	60	76	77	—	—

Absorptions of the serum were made with different adult CBA tissues. NM, unirradiated adult CBA bone marrow cell suspensions; XM, cell suspensions from the bone marrow of adult CBA mice irradiated with 400 R 12 days earlier.

Table II. Percentage of viable cells in trypan-blue cytotoxicity tests performed with NM or XM cells exposed to rabbit anti-embryonic mouse serum

Type of marrow used for additional absorption	Type of marrow tested	Percentage of viable cells						
		No serum	serum dilutions					
			1/2	1/4	1/8	1/16	1/32	1/64
NM	NM	82	84	86	82	—	—	—
	XM	80	61	57	63	60	76	84
XM	NM	82	73	82	83	—	—	—
	XM.	80	69	79	75	—	—	—

The serum was absorbed 4 times with different adult mouse tissues and, in addition, with either NM cells or XM cells.

These data suggest that the rabbit antiserum produced against embryonic mouse tissues contains antibodies which are specifically active against a part of the cell population, present in the bone marrow of irradiated mice but not in the marrow of untreated mice. Since X cells are immature in character and are present in new-born animals, it is tempting to assume that X cells which accumulate in the marrow after irradiation have specific embryonic antigens and therefore represent this fraction. However, it cannot be excluded that some part of the cell population present in irradiated marrow, X cells or another cell type, is more sensitive to certain antibodies than are unirradiated marrow cells. It is unlikely that H-2 antibodies caused the observed effect, since such antibodies are not easily detectable in xenogeneic sera[8]; the absorption procedures used would eliminate such antibodies; and, furthermore, cells from irradiated and normal bone marrow have been shown to have a similar sensitivity to the cytotoxic effect of such antibodies[9]. It is expected that further tests using immunofluorescence, and cell separation techniques will distinguish between the different possibilities discussed above[10].

Résumé. Un antisérum dirigé contre les tissus embryonnaires de la souris contient des anticorps capables de détruire sélectivement une population de cellules présente dans la moelle qui régénère après une irradiation sublétale. On peut supposer que la population détruite correspond à un type particulier de cellules lymphoïdes immatures (cellules X) qui caractérise la régénération médullaire de la souris irradiée.

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Possible Role of Growth Hormone in the Stimulation of the Thymus of Rats Following Irradiation of the Head

Exposure of the head only – with the thymus and spleen completely shielded – with doses of X-rays between 150–2000 R produces biochemical changes in the lymphoid organs of young rats[1,2]. The first effect – evident at 4 h post-irradiation and reaching a maximum at 24–48 h – is a marked increase in the rate of incorporation of tritiated thymidine in the DNA of thymus cells. In other lymphoid organs, notably the spleen, head irradiation does not affect the synthesis of DNA[2].

The object of this investigation is to examine the mechanism by which irradiation with X-rays of the head only influences DNA metabolism in the thymus. The neuro-endocrine system exerts both a negative and a positive control over the thymus. By releasing ACTH cell division is reduced via the intermediary of cortisone[3,4], on the other hand there is evidence that other pituitary hormones, growth hormone, parathyroid hormone and vasopressin may stimulate proliferation of lymphoid cells in the thymus[5–10] and growth hormone was found to be thymotropic[11]. Consequently stimulation of the thymus following head irradiation might be caused either by reducing the output of ACTH or by increasing the supply of growth hormone. In the literature data can be found in support of either mechanism after head irradiation. Mirand and Hoffman[12] and Hameed and Haley[13] deduce that the release of ACTH is reduced for a short period and Mosier and Jansons[14] find an increase in pituitary content of growth hormone. The effect of head irradiation on bilaterally adrenalectomized rats was, therefore, investigated so as to distinguish between these two mechanisms.

A further question is whether the target organ responsible for the increase in thymidine incorporation by head irradiation is the pituitary or the hypothalamus. There is