ABOLITION OF INCREASED RADIORESISTANCE OF THE IMMUNOCOMPETENT SPLEEN CELLS OF PREVIOUSLY IRRADIATED MICE BY MEANS OF SYNGENETIC LYMPHOCYTES

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Spleen cells taken from mice 14 days after irradiation in a dose of 500 R possess increased radioresistance as regards their ability to accumulate antibody-forming cells during cultivation in vivo. Addition of normal syngenetic lymphocytes to the "radioresistant" spleen cells abolished the effect of accumulation of radioresistant antibody producers. It is postulated that the increased radioresistance of immunocompetent spleen cells of previously irradiated animals depends on population changes in the interacting cell types such as are observed after exposure to ionizing radiation.

Previous investigations [6, 12] have shown that a population of spleen cells from sublethally irradiated mice possesses increased radioresistance when tested for its ability to accumulate antibody producers during cultivation in vivo. No increase in the radioresistance of the stem cells of the spleen was recorded under these circumstances [6].

To explain these facts, attention has been drawn to recent evidence regarding the role of interaction between cells of various types in immunogenesis [3, 5, 9, 11]. The present writers have suggested that increased radioresistance of immunocompetent spleen cells of previously irradiated mice depends on population changes among the interacting cell types observed after exposure to ionizing radiation. It has previously been shown that in the second or third week after sublethal irradiation, an excessive accumulation of stem cells takes place in the spleen (up to 700% of the normal population), and this is accompanied by a sharp deficiency in lymphocytes [1, 7].

The object of the present investigation was to verify this hypothesis by adding normal syngenetic lymphocytes to "radioresistant" spleen cells and then testing their resistance with respect to the accumulation of antibody-forming cells during cultivation in vivo.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice weighing 20-22 g were used. The mice donating spleen cells were irradiated in a dose of 500 R. The animals were sacrificed 14 days after irradiation, their spleen was removed, and and suspensions of spleen cells were prepared in medium No. 199 by the usual method [4]. Lymph gland cells were isolated from unirradiated mice of the same genotype. The suspensions of spleen cells prepared from previously irradiated or normal donors were irradiated with γ-rays in vivo in doses of between 100 and 800 R either separately or mixed with lymph gland cells from normal donors. The cell suspensions were injected into lethally irradiated recipients together with sheep's red cells (2×10^8). Six days after injection of the cells (at the maximum of the response) the recipients were sacrificed, their spleen was removed, suspensions of spleen cells were prepared, and the number of antibody-forming cells accumulating was determined by Jerne's method [10]. "Dose–effect" curves, which can be completely evaluated by two parameters D_0 and n [8], were plotted by the method of least squares[2].

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TABLE 1. Radiation Inactivation of Antibody-Forming Activity After Transplantation of Individual Cell Suspensions Together with 2×10^8 Sheep's Red Cells into Syngenetic Lethally Irradiated Recipients (CBA × C57BL) F_1

Number of transplanted cells	Type of cells	Number of PFC per 10^8 recipient's spleen cells, dose of irradiation (in R)					
		0	100	200	400	600	800
$25 \cdot 10^6$	Spleen cells from normal mice	2 910±288 (100)	1 455±161 (50)	803±82 (27,6)	249±31 (8,5)	52±13 (1,8)	41±8 (1,4)
$25 \cdot 10^6$	Spleen cells from 14-day irradiated donors (500 R)	115±11,4 (100)	182±27 (157)	102±14,7 (88)	126±16,6 (109)	86±14 (74,5)	29±6 (25,5)
$10 \cdot 10^6$	Ditto	97±15 (100)	121±9 (124,7)	79±21,3 (81,1)	41±25,6 (42,9)	16±5 (15,9)	9±3,8 (8,9)
$10 \cdot 10^6$	Lymph gland cells from normal mice	1 461±532 (100)	697±94 (47,7)	286±32 (19,5)	102±14 (6,9)	76±4 (5,2)	6±2,8 (0,4)
$1 \cdot 10^6$	Ditto	410±92,4 (100)	134±11,7 (33,5)	79±12,8 (19,7)	93±35,9 (23,2)	42±11,6 (10,5)	13±1,8 (3,2)

Note. Here and in Table 2, survival rate of PFC is given in parentheses (in %).

TABLE 2. Radiation Inactivation of Antibody-Forming Activity After Transplantation of Various Mixtures of Cells Together with 2×10^8 Sheep's Red Cells into Syngenetic Lethally Irradiated Recipients (CBA × C57BL) F_1

Number of transplanted cells	Type of cells	Number of PFC per 10^8 recipient's spleen cells, dose of irradiation (in R)					
		0	100	200	400	600	800
$10 \cdot 10^6$ $10 \cdot 10^6$	Spleen cells of normal mice + lymph gland of normal mice	4 670± 551 (100)	2 700± 248 (57,8)	1 454± 206 (31,1)	497± 100 (10,6)	97± 24 (2,1)	66± 26 (1,4)
$10 \cdot 10^6$ $10 \cdot 10^6$	Spleen cells of 14-day irradiated donors (500 R) + lymph gland cells of normal mice	21 123± 3 405 (100)	11 465± 1 257 (54,3)	5 531± 657 (26,2)	1 708± 263 (8,0)	308± 101 (1,5)	94± 10 (0,5)
$10 \cdot 10^6$ $10 \cdot 10^6$	Spleen cells of 21-day irradiated donors (500 R) + lymph gland cells of normal mice	8 954± 1 032 (100)	4 793± 626 (53,2)	1 862± 204 (20,7)	741± 242 (8,2)	287± 91 (3,2)	259± 68 (2,8)
$10 \cdot 10^6$ $1 \cdot 10^6$	Spleen cells of 14-day irradiated donors (500 R) + lymph gland cells of normal mice	348± 53 (100)	159± 22 (45,4)	68± 15 (19,4)	28± 6 (8,0)	38± 9 (10,8)	6± 3 (1,6)

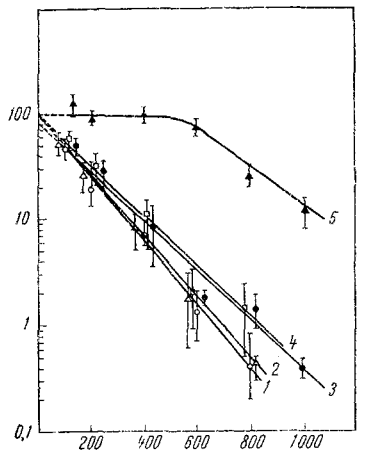

Fig. 1. "Dose–effect" curves for antibody-forming cells. Abscissa, dose of irradiation (in R); ordinate, survival rate (in percent). 1) Normal lymph gland cells; 2) mixture of normal lymph gland cells and normal spleen cells; 3) normal spleen cells; 4) mixture of normal lymph gland cells and spleen cells from 14-day irradiated donors; 5) spleen cells from 14-day irradiated donors.

The mice and cell suspensions were irradiated with Co^{60} γ-rays on a type EGO-2 apparatus (dose rate 431-517 R/min).

EXPERIMENTAL RESULTS

In the experiments of series I, "dose–effect" curves were plotted for unmixed suspensions of spleen and lymph gland cells. The results of these experiments are shown in Table 1 and Fig. 1.

As Table 1 shows, transplantation of 25×10^6 normal spleen cells together with 2×10^8 sheep's red cells led to the accumulation of about $3{,}000 \times 10^8$ plaque-forming cells (PFC) in the spleen of the lethally irradiated recipients. Irradiation of the cells in vitro before transplantation sharply suppressed plaque formation. The parameters of radioresistance were $D_0 = 188.3$ R and $n = 0.8$ respectively. Transfer of the same number of spleen cells from previously irradiated donors led to a much smaller accumulation of antibody producers (approximately (100×10^8). However, irradiation in vitro had little effect on antibody production: up to a dose of 400 R the survival rate, as shown by the number of plaque-forming cells, remained at the 100% level. The parameters of the "dose–effect" curve were $D_0 = 220$ R and $n = 10.2$. Radiation inactivation of antibody producers was similar in character when 10×10^6 spleen cells from irradiated donors were added to the culture in vivo.

The antibody-forming cells detectable after transplantation of normal lymph gland cells in a dose of 1×10^6 or 10×10^6 were inactivated in the same way by irradiation in vitro as after transplantation of normal spleen cells ($D_0 = 147$ R, $n = 0.98$).

The results of the experiments of series II, when "dose–effect" curves were plotted for various cell mixtures, are given in Table 2. The addition of 10×10^6 normal lymphocytes to 10×10^6 spleen cells from 14-day irradiated donors and the subsequent transplantation of this mixture together with sheep's red cells into lethally irradiated syngenetic recipients led to the accumulation of $20{,}000 \times 10^8$ PFC. If both components, taken in the same doses, were derived from the tissues of normal mice, the figure was $4{,}600 \times 10^8$ antibody producers. In other words, the splenic population of the 14-day donors, containing approximately 3-7 times more stem cells (CFU) than normally [1, 7], on interacting with the equivalent dose of lymph gland cells led to the accumulation of 5 times more plaque-forming cells than normal spleen cells.

The use of spleen cells from 21-day irradiated donors in the cell mixture led to the accumulation of $9{,}000 \times 10^8$ PFC.

The CFU level in the spleen at these times after sublethal irradiation was 1.5-2 times higher than normal [1, 7].

It is interesting to note that the use of 1×10^6 lymph gland cells mixed with 10×10^6 spleen cells from 14-day irradiated donors (i.e., in the ratio of 1:10) was not followed by effective interaction as regards increased accumulation of antibody producers. However, in the study of "dose–effect" curves all the mixtures tested underwent the same radiation inactivation as normal spleen cells. By the criterion of accumulation of antibody producers their radioresistance was indistinguishable from normal.

"Dose–effect" curves for the various cell mixtures are illustrated in Fig. 1. The values of D_0 and n were approximately the same in all cases.

The accumulation of "radioresistance" antibody producers observed 14 days after sublethal irradiation of the animals thus evidently reflects quantitative disturbances in cellular cooperative processes, to which a leading role is nowadays ascribed in the development of the normal immune response. Evidence in support of this conclusion is given by the fact that addition of normal syngenetic lymphocytes to the "radioresistant" population of spleen cells abolished the effect of increased radioresistance irrespective of the ratio between lymphocytes and spleen cells (1:1 or 1:10).

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