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IMMUNE RESPONSES IN AGED MICE: CHANGES OF ANTIBODY-FORMING CELL PRECURSORS AND ANTIGEN-REACTIVE CELLS WITH AGEING

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SUMMARY

The depressed immune response to sheep erythrocytes in young syngeneic irradiated recipients, adoptively restored with splenic cells from old donor mice, was used as an index of the changes in the immunocompentent cells with ageing. The numbers of antibody-forming cell precursors in both spleen and bone marrow appeared to be diminished with ageing. Some reduction of antigen-reactive cells was also observed in old spleens.

INTRODUCTION

Little is known about changes in immunocompetence with ageing. Immune responses have been reported to be depressed to extrinsic antigens (Makinodan & Peterson, 1962, 1964; Albright & Makinodan, 1966; Metcalf, Moulds & Pike, 1966; Kishimoto, Tsuyuguchi & Yamamura, 1969), but to be enhanced to intrinsic antigens with increasing age (Hildemann & Walford, 1966; Rowley, Buchanan & Mackay, 1968). The mechanisms responsible for these changes remain unknown.

In a previous communication from our laboratory, we showed that the changes in immunocompetence with ageing depend upon the antigens used. Furthermore, the reduction of the immune response to sheep erythrocyte in aged mice was found to result, not from the decreased amount of antibody synthesized by the individual cell, but from the decreased number of the antibody forming cells and their precursors (Kishimoto *et al.*, 1969).

Recently, there has been unequivocal evidence indicating that an interaction of two cell lines is required for the immune response to sheep erythrocytes in mice (Claman, Chaperon & Triplett, 1966; Mitchell & Miller, 1968; Miller & Mitchell, 1968; Nossal, Cunningham & Mitchell, 1968). One is thought to be a bone marrow-derived cell line engaging in antibody synthesis, and the other to be a thymus-dependent cell line responding to sheep erythrocytes, but not giving rise to antibody-forming cells.

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In view of these considerations, an attempt was made to determine the relationship between these two cell lines and the depressed immune response to sheep erythrocytes in aged mice. This paper presents evidence suggesting a loss of antibody-forming cell precursors in the bone marrow and the spleen and some reduction in the number of antigen-reactive cells of the spleen with ageing.

MATERIALS AND METHODS

Mice

C57BL mice of both sexes were used throughout the experiment. Their ages ranged from 2 to 19 months. Each group consisted of 7–24 mice.

Immunization procedure

Recipient syngeneic mice received intravenous transfer of $10–30 \times 10^6$ splenic or bone marrow cells, or both, from donor mice of various ages immediately after 600 r wholebody irradiation. For the preparation of bone marrow cells, excised femurs were cut at both ends, flushed with Eagle's medium and marrow plugs disrupted by aspiration through 25-gauge needles. One day after, cell transfer recipients were given 0·2 ml of a 20% sheep erythrocyte suspension intravenously.

Haemolysin plaque-forming cell titrations

The agar plaque technique devised by Jerne & Nordin (1963) was used for the detection and enumeration of 19S haemolysin-producing cells.

Enumeration of haemolytic foci

This was performed with a modification of the haemolytic focus method (Kennedy *et al.*, 1965). Recipient syngeneic mice received an intravenous injection of 10^6 nucleated splenic cells from donor mice of various ages immediately after 600 r wholebody irradiation. 1 day later, recipients were given 0·2 ml of a 10% sheep erythrocyte suspension intraperitoneally. They were killed on the 5th–8th day after immunization. The spleens were immediately frozen and sectioned approximately 300 μ thick. These sections were put, in serial order, on the sheep erythrocyte–Eagle agar plate, and incubated at 37°C for 2 hr. The agar plates were then layered with guinea-pig serum diluted 1 : 10, and the number of haemolytic foci were counted after a further 2 hr incubation.

RESULTS

A comparison was made of numbers of direct plaque-forming cells (PFC) in the spleens of 2-month-old irradiated recipients which had received 3×10^7 nucleated splenic cells from syngeneic donor mice of either 2 or 15 months of age. The number of PFC was determined on the 7th day after immunization. The number of PFC in the spleens of irradiated animals receiving cells from spleens of old mice was shown to be approximately $\frac{1}{3}$ of those obtained with young splenic cells, as indicated in Table 1. This suggests that the depressed immune response in old mice may result from the changes in the immunocytes *per se*.

To ascertain the possibility that the depressed immune response to sheep erythrocytes given by old spleen may account for the decreased number of antibody-forming cell precursors in

the old spleen, the number of haemolytic foci by Kennedy's method were estimated. A haemolytic focus is thought to consists of progeny cells arising from an antibody-forming cell precursor (AFCP) which has interacted with an antigen-reactive cell (ARC) after immunization with sheep erythrocytes. Table 2 shows that numbers of haemolytic foci in old spleens were lower than those in young spleens.

A further attempt was made to compare numbers of AFCP in the bone marrow at 2 months of age with those at 15 months of age. 10^7 bone marrow cells from syngeneic donor mice of either 2 or 15 months of age were given to irradiated recipients together with 10^7 splenic cells from donor mice of 2 months of age. Control recipients received either 10^7 bone

TABLE 1. Numbers of PFC in spleens of young syngeneic irradiated recipients, adoptively restored wth 3×10^7 splenic cells from donor mice of either 2 or 15 months of age

Age of mice	No. of mice	PFC per spleen		Statistical significance
		Geometric mean	95% confidence limits	
2 months	10	2640	3870–1800	$P < 0{\cdot}01$
15 months	10	573	1260– 260	

TABLE 2. Number of antibody-forming cell precursors in spleens of either young or old mice

Age of donor mice	No. of splenic cells transferred	No. of haemolytic foci		Statistical significance
		Individual spleen	Mean ± SE	
2 months	10^6	0, 0, 1, 1, 1, 2, 3, 3, 5	1·8 ± 0·5	$P = 0{\cdot}05$
15 months	10^6	0, 0, 0, 0, 0, 1, 1, 1, 2	0·6 ± 0·2	

marrow cells or splenic cells alone. Numbers of PFC were determined on the 7th–8th day after immunization with sheep erythrocytes when the peak number of PFC was expected to occur. Recipients of a mixture of young splenic and young bone marrow cells had much more PFC in their spleens than can be accounted for by simple summation of numbers of PFC in those of either cells alone. Those of a mixture of young splenic and old bone marrow cells had no more PFC than the sum of numbers of PFC in those of either cells alone, as indicated in Table 3. This suggests that old bone marrow was more depleted of AFCP than young bone marrow.

Antigen-reactive cells are known to be required for AFCP to proliferate and differentiate to antibody-forming cells (AFC) in the response of mice to sheep erythrocytes. To compare numbers of ARC in the old spleens with those in the young ones, irradiated recipients received an intravenous injection of 10^7 splenic cells from donor mice of either 2 or 15 months of age together with 10^7 bone marrow cells from donor mice of 2 months of age. Control mice were given either bone marrow or splenic cells alone. On the 7th–8th day after

immunization, they were killed for PFC studies on the spleens. The results in Table 4 show that the synergistic effect on the number of PFC was observed in recipients of young bone marrow and splenic cells irrespective of their ages. However, the numbers of PFC in recipients of both young bone marrow and old splenic cells appeared little more than the sum of those in recipients of either cells alone. This seems to suggest that the number of ARC is less in the old spleens than in the young ones.

TABLE 3. Numbers of PFC in spleens of irradiated recipients of either young or old bone marrow with or without young splenic cells

Cells transferred			PFC per spleen		
Spleen	Bone marrow	No. of mice	Geometric mean	95% confidence limits	Statistical significance
10^7, young	—	16	338	570–200	
—	10^7, young	24	78	97– 63	
—	10^7, old	9	99	213– 46	
10^7, young	10^7, young	9	1420	2440–831	$P<0.01$
10^7, young	10^7, old	9	393	729–212	
—	—	14	9	21– 3	

TABLE 4. Numbers of PFC in spleens of irradiated recipients of either young or old splenic cells with or without young bone marrow

Cells transferred			PFC per spleen		
Spleen	Bone marrow	No. of mice	Geometric mean	95% confidence limits	Statistical significance
—	10^7, young	24	78	97– 63	
10^7, young	—	7	394	575–270	
10^7, old	—	7	170	250–115	
10^7, young	10^7, young	10	1134	1415–916	$P<0.05$
10^7, old	10^7, young	10	496	604–399	

DISCUSSION

A haemolytic focus estimated by the method of Kennedy *et al.* (1965) is thought to arise from progeny cells of an AFCP which has interacted with an ARC responding to sheep erythrocytes (Shearer & Cudkowicz, 1968). Accordingly, a decrease in numbers of haemolytic foci in old spleens indicates reduction of AFCP in old spleens in contrast with young spleens. Since AFCP in the spleens are also known to come from bone marrow (Linna & Liden, 1969) which contains no ARC but AFCP alone (Miller & Mitchell, 1968), numbers of AFCP in the bone marrow were assayed by the use of the synergistic effect on the production of PFC between splenic cells and bone marrow. This synergism between splenic cells and bone

marrow has been reported by Talmage, Radovich & Hemmingsen (1969) who showed that some of PFC were derived from donor spleens and the remainder from donor bone marrow in irradiated recipients of both bone marrow and spleens, by the use of antiallotype sera. An increase of about 1000 PFC was found in the spleens of irradiated recipients of both young marrow and young splenic cells over the simple sum of the number of PFC in those of either young splenic cells or young marrow alone. These PFC were considered to be derived from AFCP present in young bone marrow. No such increase in the number of PFC in the spleens of irradiated recipients of both old bone marrow and young splenic cells was noticed. This is probable evidence for a lack or pronounced reduction of AFCP in old bone marrow.

In the experiment to assay ARC in the spleens, synergism between young bone marrow and either old or young splenic cells was observed. However, the increase (248) in the number of PFC in the spleens of irradiated recipients of a mixture of old splenic cells and young bone marrow was less than that (662) in those of both young splenic cells and young bone marrow. This may be interpreted as indicating that some ARC exists in old spleens, but less than in young spleens. The more striking reduction of AFCP in the spleens and bone marrow compared with that of ARC in the spleens with ageing may agree with the fact that AFCP derived from bursa-equivalent origin are short-lived, whereas ARC derived from thymic origin are known to be long-lived (Roitt *et al.*, 1969).

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ABBREVIATIONS

PFC direct plaque forming cells
AFCP antibody-forming cell precursors
ARC antigen-reactive cells
AFC antibody-forming cells