

Increased Content of Cells Producing Antibodies against Syngeneic Erythrocytes in the Spleen of C57Bl/6 Mice as a Result of Heterochronous Parabiosis

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The effect of lymphocyte exchange between young and old C57Bl/6 mice in parabiotic pairs on the formation of autoreactive antibody-producing cells is studied during primary immune response. It is found that the content of cells producing antibodies against bromelain-treated murine erythrocytes after immunization with sheep erythrocytes is almost the same in young and old animals (as well as in the members of age-matching parabiotic pairs). However, parabiosis between young and old animals suppresses the immune response to sheep erythrocytes in young mice and stimulates the immune response to bromelain-treated murine erythrocytes both in young and old mice.

Key Words: aging; parabiosis; autoimmunity

A marked decline of immune response during aging has been observed against the background of unchanged or even increased total production of immunoglobulins. Antigen administration was shown to induce the formation of considerable amounts of plasma cells in the spleen of old C57Bl/6 mice in comparison with young mice, the proportion of the antigen-specific antibody-producing cells (APC) being markedly decreased, while that of nonspecific APC being markedly increased [1]. The majority of nonspecific APC is represented by cells producing "naturally" occurring polyreactive anti-IgM antibodies capable of binding to a wide range of autoantigens [6]. Involution of the thymus followed by alterations of the composition of peripheral T cells probably causes the age-related decline of the control over the activation of nonspecific (including autoreactive) APC. In a model of T cell transfer between irradiated syngeneic animals it was de-

monstrated that T cells of old donors can impart some traits of the immune response of old animals to B cells of young recipients [7]. This study is an attempt to examine the effect of lymphocyte exchange between young and old animals in parabiotic pairs on the formation of autoreactive APC during the primary immune response.

MATERIALS AND METHODS

Experiments were carried out on young (3 months) and old (18 months) female C57Bl/6 mice. Parabiotic pairs were obtained as described elsewhere [3]: the abdominal cavity of each partner was open, and the peritoneal walls were sutured, after which the scapulae and skin flaps were connected. Functional vascular anastomoses formed by day 4 after surgery. Two months later, the blood exchange half-time was 2.5 h. Experiments were performed 3 months after surgery. Four days prior to the experiment, the animals were immunized with sheep erythrocytes (1×10^8 cells, intraperitoneally). The number of specific APC in the

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spleen was determined in the reaction of local hemolysis in gel [5]. In order to reveal autoreactive APC, mouse erythrocytes treated with bromelain [1] were added together with spleen cells to 0.5% agarose when the local hemolysis reaction was performed. Bromelain increases the number of "natural" antibody-binding determinants on the erythrocyte surface. The content of Thy1.2⁺ and CD4⁺ cells was determined in the complement-dependent cytotoxic test with the use of culture media conditioned by HO-13-4 and RL-174.2 hybridomas producing IgM against the correspondent T cell marker. The hybridomas were kindly supplied by Prof. K. Hirokawa (Tokyo). Immune responses were compared using bilateral Wilcoxon's *U* test, and the Wilcoxon *T* tests was used for paired samples. The survival data were analyzed using Fischer's exact test.

RESULTS

Previously, we showed that in parabiotic pairs of CBA/Ca mice the immune system of the young partner ages prematurely [2]. This is confirmed by the present study. The immune response to sheep erythrocytes (SE) significantly ($p < 0.05$) dropped in the young partners of heterochronous pairs (Fig. 1), while the relative weight of the thymus decreased and that of the spleen increased in comparison with young mice in homochronous pairs. In contrast to CBA mice, who better tolerated the parabiosis surgery, the immune response in young C57Bl/6 mice in homochronous pairs slightly dropped compared with that in intact controls (difference not significant). In heterochronous pairs, an asymmetric distribution of specific APC was observed: although the content of these APC in the spleen of the young partner dropped, it was significantly ($p < 0.05$) higher in the spleen of the old partner. This may be indicative of a stimulatory effect of the microenvi-

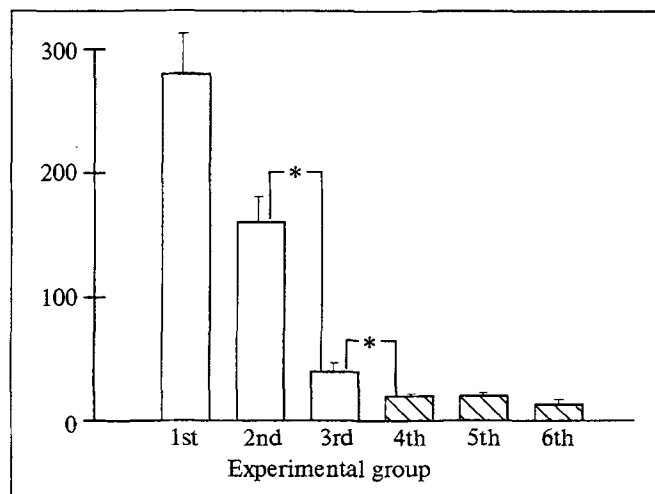


Fig. 1. Primary immune response to SE in young and old parabiotic mice. Ordinate: content of APC per 10⁶ splenocytes. Groups are designated as in Table 1. Here and in Figs. 2 and 3: shaded bars show the immune response in old mice. Statistically significant differences are indicated with asterisk.

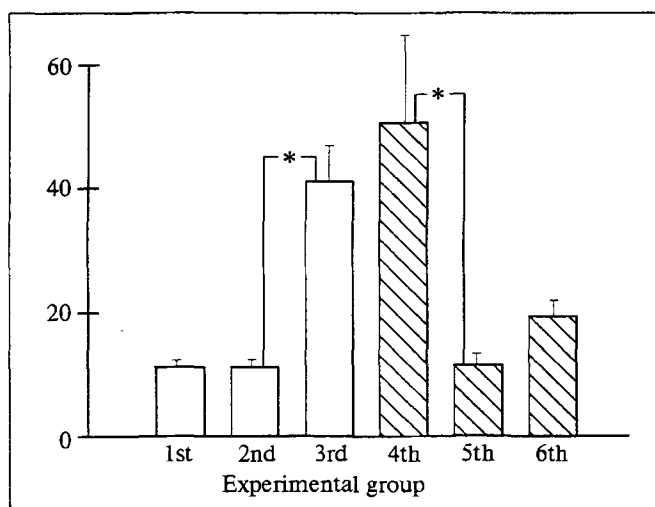


Fig. 2. Content of cells producing antibodies against syngeneic bromelain-treated erythrocytes after immunization with SE. Ordinate: content of autoreactive APC per 10⁶ splenocytes.

TABLE 1. Thymus and Spleen Indexes of Parabiotic Mice ($M \pm m$)

Experimental group	Relative mass, mg/g body weight		Lymphocyte content in the spleen, %	
	thymus	spleen	Thy1.2 ⁺	CD4 ⁺
1st	2.00±0.16 (15)	4.07±0.20 (15)	34.0±0.9 (9)	14.3±0.6 (9)
2nd	1.99±0.20 (22)	3.75±0.15 (22)	35.7±1.0 (12)	16.2±1.5 (12)
3rd	0.77±0.13* (8)	5.27±0.83* (8)	38.3±3.5 (4)	20.3±2.6* (4)
4th	0.73±0.17 (8)	3.45±0.25 (8)	37.8±2.8 (8)	16.5±1.4 (8)
5th	0.56±0.13 (12)	3.77±0.30 (12)	44.0±3.8** (8)	16.1±1.4 (8)
6th	0.48±0.04* (15)	4.00±0.27 (15)	42.3±2.9* (8)	18.1±1.0* (8)

Note. Experimental groups: 1st contains young intact mice; 2nd — young members of homochronous parabiotic pairs, 3rd — young partners of heterochronous pairs, 4th — old partners of parabiotic pairs, 5th — old members of homochronous parabiotic pairs, and 6th — old intact mice. The number of mice is indicated in parentheses. $p < 0.05$: *compared with the 1st group, **compared with the 2nd group.

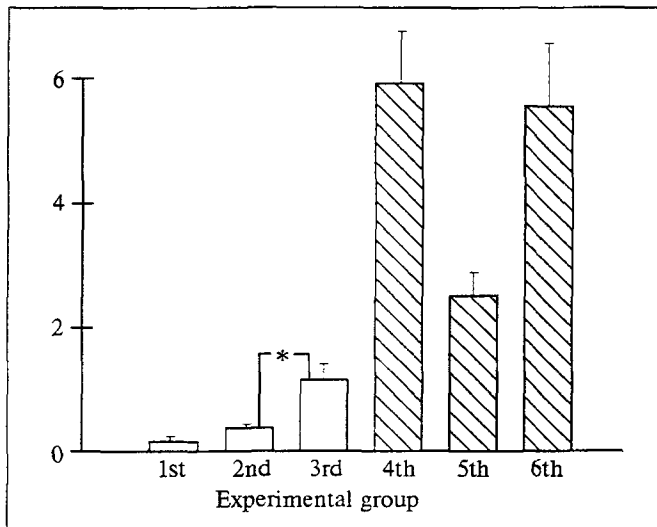


Fig. 3. Ratio between the contents of autoreactive and specific APC after immunization with SE. Ordinate: ratio between the number of autoreactive APC (per 10^6 splenocytes) and the number of APC against SE (per 10^6 splenocytes).

ronmental factors of young spleen (Fig. 1). Study of the autoimmune component coincident with the response to SE showed that the levels of formation of APC against autologous erythrocytes are similar in young and old mice as well as in parabiotic homochronous pairs. On the other hand, a significant ($p < 0.05$) increase in the content of autoreactive APC was observed in both partners of a heterochronous pair (Fig. 2). The ratio between the levels of autoreactive and specific APC after immunization with SE is worth considering. As shown in Fig. 3, during the primary immune response in young intact animals (and in parabiotic pairs of young mice) the ratio ranges from 0.1 to 0.2, i.e., the for-

mation of specific APC markedly (5- to 10-fold) exceeds that of autoreactive APC. In intact and parabiotic old mice, this ratio is greater than 1, indicating the prevalence of a nonspecific component of the immune response. An increase in the content of autoreactive APC in heterochronous pairs (and its absence in homochronous pairs) may be due to different mechanisms inhibiting the activation of autoreactive B cells in young and old animals. In heterochronous parabionts, the difference in the lymphocyte repertoire of young and old mice may manifest itself in a conflict similar to a chronic graft versus host reaction (GVHR), which is characterized by polyclonal activation of the recipient's B cells by donor's $CD4^+$ cells. An increase in the relative mass of the spleen of the young partner and elevated content of $CD4^+$ splenocytes in the old partner may indicate the development of GVHR (Table 1). This reaction was previously demonstrated in parabiotic pairs of syngeneic animals [4]. Parabiosis between young and old animals markedly lowers the 3-month survival of young partners. The mortality rate was higher in heterochronous pairs ($p = 0.005$) than in young homochronous pairs (Table 2). Thus, parabiosis between young and old C57Bl/6 mice inhibits the formation of specific APC in young partners and stimulates the formation of autoreactive APC in both partners, which probably points to age-related differences in the functioning of mechanisms controlling autoreactive clones.

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TABLE 2. Survival of Parabites

Type of parabiotic pair	Number of pairs		Significance level
	survived	died	
Young partners	14	6	-
Young and old partners	12	28	$p = 0.005^*$
Old partners	11	16	$p = 0.076^*$

Note. *Compared with young homochronous pairs.

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