

## Effects of immunoagents on circulating serum hemolysin formation in bone marrow and spleen<sup>1</sup>

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**ABSTRACT** The bone marrow as a source of serum hemolysin to sheep red blood cells (SRBC) was studied in splenectomized mice. Splenectomy prevented the hemolysin formation in the primary response, but not in the secondary response. Cyclophosphamide 100 mg/kg and dexamethasone 10 mg/kg decreased hemolysin formation in the bone marrow as well as in the spleen. Levamisole 50 mg/kg increased its formation in both organs. Prednisolone 10 mg/kg significantly suppressed its formation in the spleen, but not in the bone marrow. Hydroxyurea 50 mg/kg suppressed its formation in the bone marrow, but not in the spleen. These results suggest that the bone marrow is the major source of serum hemolysin to SRBC during the secondary response and drug has different effects on antibody production in the bone marrow and in the spleen.

**KEY WORDS** hemolysins; bone marrow; spleen; dexamethasone; cyclophosphamide; levamisole; prednisolone; hydroxyurea

Over past years the concept of the bone marrow as the major source of antibody has not been widely accepted<sup>(1,2)</sup>, for the bone marrow of mice being the most popular experimental animals in immunological research showed few or even no antibody formation cells after immunization<sup>(1,3)</sup>. However, some activity in the bone marrow was found with an increasing interval after immunization with SRBC<sup>(4)</sup>. The number of cytoplasmic Ig-positive plasmablasts, plasma cells and Ig-secreting cells in the bone marrow was substantial as compared with that in the other

lymphoid organs, which suggested that the bone marrow might be capable of producing antibody under conventional conditions<sup>(5,6)</sup>. It was reported that corticosteroid enhanced the bone marrow IgG production in patients with idiopathic thrombocytopenic purpura and the bone marrow plaque-forming cells (PFC) to lipopolysaccharides in mice<sup>(7)</sup>. The contribution of the bone marrow to serum hemolysin of SRBC and different effects of drugs on antibody formation in the bone marrow and in the spleen, which is little known, were studied in this paper.

### MATERIALS AND METHODS

**Animal and drugs** NIH mice, ♂, 22 ± SD 2 g, were obtained from Zhejiang supply center of experimental animals. Mice were anesthetized by ip pentobarbital sodium 50 mg/kg, then the spleen was removed. There was no post-operative death. Cyclophosphamide (200 mg/vial) and prednisolone (125 mg/5 ml) were purchased from Xianju Pharmaceutical Factory, dexamethasone (5 mg/ml) and levamisole (200 mg/vial) were from Zhejiang Medical University, and hydroxyurea (500 mg/vial) was made by Ji-nan Pharmaceutical Factory, Ji-nan, China.

**Antigen and immunization** SRBC taken from a single sheep were collected in Alsever's solution and stored at 4°C. They should be used within 1-2 wk. For the primary or secondary response to SRBC, mice were immunized with ip 20% SRBC ( $2 \times 10^9$ /ml) 0.2 ml/mouse.

**Determination of serum hemolysin to SRBC** Hemolysin was determined as des-

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cribed previously<sup>(8)</sup>.

**Statistics** The results were expressed as  $\bar{x} \pm SD$ . Statistical analyses were calculated by the *t* test.

**RESULTS**

**Primary response to SRBC** In the primary response to SRBC injected on d 0, the time-course of hemolysin production in mice was shown in Fig 1. The level of serum hemolysin remained undetectable or very low until d 2, peak response was achieved on d 5 and then declined gradually. Serum hemolysin could not be observed after 4 wk.

Independent of the antigen dose, peak hemolysin level was always found on d 5 after immunization (data not shown).

Splenectomy was performed on d 10 before primary injection of SRBC. In the splenectomized mice, the production of serum hemolysin could be partially or even completely prevented (Fig 1), which suggested that serum hemolysin to SRBC during the primary response should be derived from the spleen.

**Effects of 5 drugs on serum hemolysin from spleen** As shown in Tab 1, the hemolysin concentration in mice was decreased by dexamethasone, cyclophosphamide or prednisolone, but increased by levamisole. This observation corresponded well with the other results published<sup>(8-10)</sup> Effect of

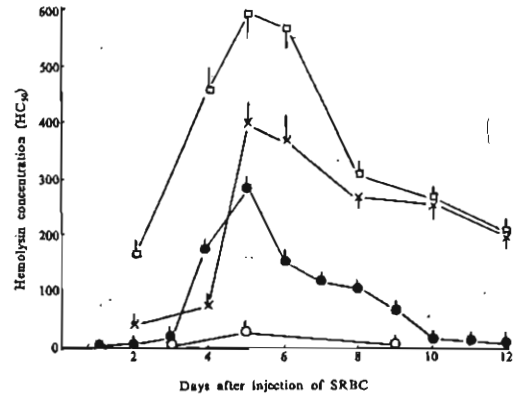


Fig 1. Kinetics of serum hemolysin formation during primary (O, ●) and secondary response (×, □). (O) Splenectomy on d 10 before injection of SRBC. (×) Splenectomy on d 25 after primary injection of SRBC, second injection of SRBC given on d 35 after first one. (●) and (□) normal mice. *n* = 5,  $\bar{x} \pm SD$ .

hydroxyurea on hemolysin was not observed.

**Secondary response to SRBC** A second SRBC ip on d 35 after the first one, resulted in the rapid appearance of serum hemolysin (Fig 1). Hemolysin level during the secondary response was much higher than that during the primary response.

It was reported that during the secondary response a very distinct PFC activity was found in the bone marrow and in the spleen, but not in the peripheral lymph nodes, the

Tab 1. Effects of 5 drugs on serum hemolysin formation in spleen and bone marrow. *n* = 10,  $\bar{x} \pm SD$ . \**P* > 0.05, \*\**P* < 0.05, \*\*\**P* < 0.01 vs control.

Pretreatment	Dose (mg / kg)	Spleen hemolysin <sup>+</sup> Route & Days	Bone marrow hemolysin <sup>++</sup>	
			Hemolysin (HC <sub>50</sub> )	Route & Days Hemolysin (HC <sub>50</sub> )
Control	0		294 ± 46	391 ± 47
Levamisol	50	po, d 0 to d 3	394 ± 69***	po, d 35 to d 38 436 ± 37*
Cyclophosphamide	100	ip, d 1	18 ± 9***	ip, d 36 20 ± 12***
Dexamethasone	10	sc, d 0 to d 2	92 ± 27**	sc, d 35 to d 37 329 ± 60**
Prednisolone	10	sc, d 0 to d 2	164 ± 33***	sc, d 35 to d 37 358 ± 65*
Hydroxyurea	50	ip, -d 1	315 ± 52*	ip, d 34 317 ± 47**

<sup>+</sup> Mice were immunized with 20 % SRBC (0.2 ml / mouse, ip) on d 0, hemolysin was determined on d 5 after immunization. <sup>++</sup> Mice were splenectomized on d 25 after primary injection of SRBC, 2nd injection of SRBC was given on d 35 after the 1st one, hemolysin was determined on d 7 after 2nd injection of SRBC.

mesenteric lymph nodes and Peyer's patches<sup>(11)</sup>. To find out whether or not serum hemolysin would come from the bone marrow during the secondary response in our system, mice were splenectomized on d 10 before the second injection of SRBC. Fig 1 showed splenectomy was unable prevent serum hemolysin production. During the first 5-10 d, serum hemolysin predominantly came from the bone marrow, from d 10 the bone marrow became an unique source of hemolysin. It was concluded that formation of serum hemolysin to SRBC in the secondary response mainly occurred in the bone marrow.

On the other hand, splenectomy prior to primary injection of SRBC could reduce serum hemolysin production during the secondary immune response (data not shown).

**Effects of 5 drugs on serum hemolysin from bone marrow** The hemolysin concentration in mice was decreased by cyclophosphamide, dexamethasone or hydroxyurea, but increased by levamisole. In prednisolone-treated mice, it was not significantly decreased (Tab 1).

## DISCUSSION

It is remarkable that the bone marrow considered to be a central lymphoid organ has never been generally accepted as a site of antibody formation, in contrast to the spleen<sup>(1-3)</sup>. This may be due to the fact that many studies in which the bone marrow was investigated as a site of antibody formation yielded negative results<sup>(12)</sup>. However, the present experiment provided a contrary evidence that the bone marrow was actually the major source of circulating serum antibody to SRBC during the secondary response although the spleen seemed to be the only site of antibody production during the primary response.

The mechanism underlying antibody formation in the bone marrow still remains unknown. According to the results, the bone marrow antibody production could be reduced

by splenectomy prior to the the primary immunization, but not prior to the secondary immunization, which revealed that the spleen was involved in the antibody formation in the bone marrow. The most plausible explanation is that antibody formation in the bone marrow is dependent on the B memory cells of peripheral lymphoid organs migrating into the bone marrow, a subsequent antigenic stimulation triggers the development of these cells into antibody formation cells and produce large amount of antibody. The results of the others have supported such an explanation<sup>(13-15)</sup>

When one becomes old, more antigenic stimulation from the environment will have been experienced and secondary-type response will prevail, the importance of the bone marrow as a site of antibody synthesis throughout the lifespan increases gradually. We consider it worthwhile to study drugs regulating antibody production in the bone marrow. We found that antibody production in the bone marrow was rather resistant to prednisolone or dexamethasone mediated suppression and that hydroxyurea significantly suppressed antibody formation in the bone marrow, but not in the spleen. Now, experiments in the mechanism underlying different effects of drug on antibody formation in the bone marrow and in the spleen are being carried on.

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**免疫药物对骨髓和脾脏产生循环血清溶血素的影响。**

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**提要** 用去脾小鼠观察骨髓能否作为血清抗绵羊红细胞溶血素的产生场所。抗原免疫前切脾可抑制初次溶血素应答而不能抑制再次溶血素应答。环磷酰胺(100 mg/kg)和地塞米松(10 mg/kg)抑制骨髓和脾脏溶血素产生,左旋咪唑(50 mg/kg)可增强溶血素产生;泼尼龙(10 mg/kg)抑制脾脏溶血素产生而对骨髓溶血素产生无显著影响;相反,羟基脲(50 mg/kg)抑制骨髓产生溶血素,而对脾脏产生溶血素无影响。结果表明骨髓是第二次免疫应答中血清溶血素的主要来源,某些药物对骨髓和脾脏产生抗体有不同的影响。

**关键词** 溶血素;骨髓;脾脏;地塞米松;环磷酰胺;左旋咪唑;泼尼龙;羟基脲

**重 要 消 息**

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