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Suppression of IgM-producing Cells by Administration of Anti-IgM Serum *in Vivo* before Sheep Red Blood Cells Injection

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The primary immune response in young adult mice to sheep red blood cells was studied by following the effects of anti-IgM or anti-Fc sera on the number of plaque-forming cells for IgM (dPFC) and IgG (iPFC) in the spleen. The number of dPFC in the case of administration of anti-IgM serum at 1 h before antigen injection was significantly decreased to $3.7 \pm 0.9/10^6$ spleen cells in comparison with the value of control serum ($69.0 \pm 15.7/10^6$ spleen cells). However, the numbers of iPFC in the cases of both anti-IgM or control sera were similar. From these results, we suppose that progenitors of IgM-producing cells are different from progenitors of IgG-producing cells in adult mice.

Keywords——primary immune response; suppression of IgM-PFC; anti-IgM; anti-Fc; IgM-producing cells; IgG-producing cells; differentiation of lymphocytes

Introduction

In the primary immune response of adult mice immunized with sheep red blood cells (SRBC), the peak of the number of IgM-producing cells was on day 4 and that of IgG-producing cells was on day 6.[1] Two mechanisms for this difference can be considered, as shown in Fig. 1. The first is that these IgM-producing cells and IgG-producing cells are developed from an identical progenitor cell (Fig. 1B); this mechanism is described in an immunological textbook.[2] This first mechanism is supported by the findings that IgM-producing cells, on being micromanipulated, developed to IgG- or IgA-producing cells, and some myeloma cells producing IgM changed to IgG-producing cells.[3–5] The second is that IgM-producing cells and IgG-producing cells are developed from two kinds of separate progenitor cells, previously differentiated during their maturation (Fig. 1A). We studied the feasibility of

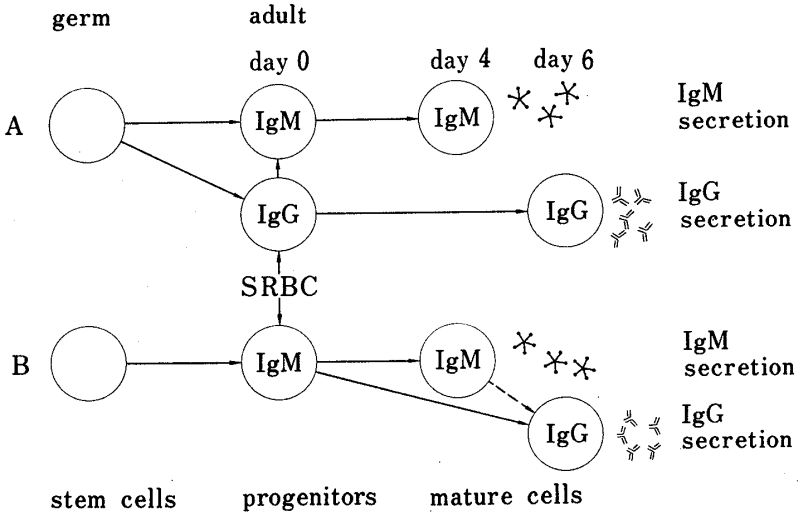

Fig. 1. Two Possible Mechanisms of the Primary Immune Response of Adult Mice to SRBC

The results in this report support model A.

this mechanism by *in vivo* administration of anti-IgM or anti-Fc sera, depending on the basic phenomenon of *in vitro* suppression of IgM PFC with anti-IgM serum.[6]

Meanwhile, many experiments with anti-immunoglobulin sera have been done. In chickens, administration of anti-IgM *in ovo*-suppressed the synthesis not only of IgM but also of IgG, so IgG-producing cells must arise from identical cells having surface IgM.[7] *In vivo* administration of anti-IgM serum to neonatal mice resulted in a polyclonal suppression of B cells stimulatable to IgM, IgG or IgA secretion.[8,9] It has also been shown that administration of anti-δ enhanced humoral immunity depending on the stimulation of T cells by anti-δ.[10]

Experimental

Preparation of Anti-serum——The mouse strain used was ICR, from whose serum IgG and IgM were purified by fractional precipitation with ammonium sulfate and gel filtration on Sephadex G-200. Anti-IgM serum was obtained from an albino rabbit immunized with IgM (a fraction that passed through a Sephadex G-200 column) emulsified in Freund's complete adjuvant. Contaminating antibodies such as anti-IgG in anti-IgM serum were removed by absorption with neonatal mouse serum. The absence of anti-IgG contamination in anti-IgM serum was confirmed by immunoelectrophoresis.

IgG fraction, obtained from a Sephadex G-200 column, was digested with papain and chromatographed on DEAE-cellulose. Fc fraction obtained from DEAE-cellulose column chromatography was injected together with Freund's complete adjuvant into a rabbit in order to prepare anti-Fc serum. Some of the anti-IgM and anti-Fc used in this experiment were supplied by the Medical Biological Laboratory, Midori-ku, Nagoya Japan.

PFC Assay——Assay of direct plaque-forming cells (dPFC), which are IgM-producing cells, was carried out by the soft agar method of Jerne and Nordin.[11] The number of indirect plaque-forming cells (iPFC), which are IgG-producing cells, was counted from haemolytic plaque originated from anti-Fc and complement after prevention of dPFC with anti-IgM serum.[6] The numbers of dPFC and iPFC per 10^6 spleen cells were measured on day 6 after intraperitoneal injection of 20% SRBC (0.4 ml/10 g of mouse body weight). Anti-IgM serum or anti-Fc serum (0.2 ml/adult mouse; 0.05 ml/neonatal) was injected in the same place (IP) as antigen, before or after the antigen administration in 5-week-old adult mice (one half of the littermates). The other half of the littermates was injected with control serum not containing antibody to mouse IgM and IgG. Neonatal mice were injected within 12 h after birth.

Results and Discussion

Table I shows the results of antibody-mediated suppression of dPFC. The number of dPFC in the case of administration of anti-IgM serum to 5-week-old adult mice at 6 h before antigen injection was 31.7 ± 11.9, and this value was significantly different from the number (89.6 ± 3.1) in the case of control serum. However, the number of iPFC in the cases of anti-IgM serum and control serum were similar (265 and 255, respectively). This suppression of dPFC with anti-IgM serum was clearly seen on administration of the serum at 1 h before injection of SRBC. The number of dPFC in the case of administration of anti-IgM serum at 1 h before antigen injection was clearly decreased (3.7 ± 0.9) compared with the value of control serum (69.0 ± 15.7). Stronger suppression of anti-IgM serum at 1 h than at 6 h supports the review that this suppression might be caused by covering the surface IgM on B cells and macrophages with anti-IgM, as in the *in vitro* suppression of dPFC with anti-IgM. In the case of administration of anti-IgM serum at 6 h (results for two experiments are shown in Table I), the anti-IgM antibody injected intraperitoneally must diffuse to peripheral areas and be processed by phagocytic cells before contacting the antigen SRBC, so the difference between the values of anti-IgM and the control was lower than that in the case of administration of anti-IgM serum at 1 h before the antigen injection.

Administration of anti-IgM serum at 1 h after SRBC injection did not influence the number of dPFC. Administration of anti-Fc serum before SRBC injection did not suppress the number of dPFC and iPFC as shown in Table I. The large difference of iPFC number between experimental groups in the case of anti-IgM administration, as shown in Table I, may be due to differences of SRBC (aging of SRBC), but we have no evidence. However, all the cases

Table I. The Influence of Anti-IgM and Anti-Fc Sera on the Number of PFC

Mouse	Serum	Interval: Before SRBC injection	Interval: After SRBC injection	(n)	PFC/10^6 spleen cells (mean ± S.E.): Direct (IgM)	PFC/10^6 spleen cells (mean ± S.E.): Indirect (IgG)
Adult (5-week-old)	Anti-IgM	1 h	—	5	3.7± 0.9[a]	14.2± 1.6
	Control	1 h	—	5	69.0±15.7	16.0± 2.4
	Anti-IgM	1 h	—	4	15.8± 2.6[a]	27.4± 5.9
	Control	1 h	—	6	70.2±12.7	28.1± 3.0
	Anti-IgM	6 h	—	6	31.7±11.9[a]	265± 9.5
	Control	6 h	—	6	89.6± 3.1	255±14.9
	Anti-IgM	6 h	—	6	51.4± 6.4[a]	183±26.4
	Control	6 h	—	6	81.6± 5.6	121±17.9
	Anti-Fc	6 h	—	6	294±78	132±33
	Control	6 h	—	6	226±31	118±63
	Anti-IgM	—	1 h	6	59.9± 3.3	18.6± 5.5
	Control	—	1 h	6	29.4± 3.6	5.6± 0.4
Neonatal[b]	Anti-IgM	5 weeks	—	5	33.0± 7.1	18.2±16.8
	Anti-Fc	5 weeks	—	4	40.5±14.0	41.2±18.5
	Control	5 weeks	—	9	56.2±10.8	59.8±23.8

a) Significantly different from control: $p < 0.01$.
b) Two littermates were used.

of anti-IgM administration before SRBC injection in Table I indicate that the iPFC numbers were not influenced by anti-IgM.

It was shown that progenitors of IgG-producing cells carried surface IgM and surface IgD.[12] Development of the progenitors to IgG-producing cells was not suppressed by anti-Fc serum in this work. The role of surface IgD on IgG progenitors for development to IgG-producing cells should be studied.[10]

Using neonatal mice, the results of suppression of dPFC and iPFC with anti-IgM serum are also shown in Table I. Administration of anti-IgM serum in neonatal mice suppressed the number of iPFC as well as dPFC. Anti-Fc serum did not show distinct suppression. These results are coincident with those obtained in a study with mice.[8,9]

The treatment with anti-IgM serum in the adult stage did not suppress iPFC but suppressed dPFC. These results suggest that stem cells having surface IgM in the neonatal period differentiate to separate IgM-producing cells and IgG-producing cells during maturation after birth. In adult mice, progenitors of IgG-producing cells and IgM-producing cells present independently from each other (case A in Fig. 1). IgG-producing cells did not differentiate from progenitors of IgM-producing cells after the primary SRBC injection but might have developed from progenitors of IgG-producing cells. The antibody genes in adult lymphocytes were also different from those of murine embryo.[13]

In the primary immune response, dPFC showed the greatest increase on day 4 and iPFC on day 6.[1] This difference may be due to a difference of generation time and/or in the number of IgM-progenitors and IgG-progenitors. In the secondary response, the number of memory B cells producing IgG is greater than that of cells producing IgM, and humoral IgG in serum is produced to a greatest extent than IgM. On the basis of our view of different progenitors of IgM- and IgG-producing cells, the case of production of only IgG or only IgM upon primary stimulation can be accounted for. It is possible that some immune deficiency diseases are a result of a defect in differentiation to each progenitor cell from stem cells.

We used humoral murine serum as a source of antigen for the preparation of rabbit anti-IgM serum. Recently it has been reported that surface IgM has a different carboxyl-terminal from humoral IgM.[14] However, this difference may be minor, because anti-humoral IgM serum suppressed the development of IgM-producing cells, which carry surface IgM. Although

our results support model A in Fig. 1, our experiments contain some factors to lead to the erroneous supposition. These points are the *in vivo* administration of rabbit serum containing many kinds of proteins, the relation between the doses of antiserum and SRBC, and the low number of iPFC on the primary response. Therefore, the conclusion is tentative and should be confirmed by the further study, as well as the roles of T cells and macrophages should be investigated.

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