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Effects of splenectomy on the humoral immune system

A STUDY IN NEONATALLY AND ADULT SPLENECTOMIZED MICE

J. ROZING, N. H. C. BRONS & R. BENNER *Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands*

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Summary. Experiments were performed to investigate the influence of neonatal and adult splenectomy on humoral immunity in mice. In the bone marrow and lymph nodes of both groups of splenectomized mice the number of immunoglobulin (Ig)-positive (B) lymphocytes was significantly higher than in sham-operated mice. These higher numbers of B cells probably reflect a compensation for the absence of the B cell population of the spleen. Hardly any quantitative differences in the serum immunoglobulins were found between splenectomized and sham-splenectomized mice. Only for the IgM class was a significantly lower concentration found in the serum of splenectomized animals. This low concentration of IgM in the blood of splenectomized mice was caused by a failure of the remaining organs to compensate completely for the removal of the quantitatively important population of IgM-producing plasma cells in the spleen. Nevertheless, the number of precursors of IgM-producing plasma cells in bone marrow and lymph nodes and their ability to differentiate into IgM-producing plasma cells was not diminished by splenectomy. Probably the spleen provides a highly efficient environment for the differentiation into IgM-producing plasma cells.

By investigating the synergistic ability of bone marrow cells and thymus cells from neonatally splenectomized mice it was found that these cells were fully capable of co-operating in the adoptive plaque-forming cell response to sheep red blood cells (SRBC).

Correspondence: Dr J. Rozing, Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, Rotterdam, The Netherlands.

INTRODUCTION

It is remarkable that an individual can survive the removal of such an important organ as the spleen. In mice the spleen has in addition to its involvement in the regulation of the circulation of blood and the production of cells of the erythroid and granuloid series, an important role in the immune defence, especially against blood born viral and bacterial infections (Weiss, 1972). The fact that splenectomy nevertheless in general does not cause any severe post-operative complications, suggests the existence of strong compensatory mechanisms for the loss of the various functions of the spleen.

However, various effects of removal of the spleen have been reported. These influences are mostly related to functions of the spleen in the immune system. Although no effect of splenectomy can be demonstrated on the primary allograft rejection (Pierce & Hume, 1968; Souther, Morris & Vistnes, 1974), the hyperacute secondary allograft rejection as found in secondary kidney transplantation in man can be prevented by splenectomy before or during

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the primary transplantation (Pierce & Hume, 1968). Furthermore, it has been reported that splenectomy affects the concentration of serum immunoglobulins, in particular the IgM class (Kalpaktsoglou & Good, 1973; Claret, Morales & Montaner, 1975) and evokes a long-lasting lymphocytosis (Pedersen & Videbaek, 1966) predominantly consisting of B cells (Rozing, Brons & Benner, 1977a). Furthermore, it has been found in splenectomized, irradiated and foetal liver reconstituted mice, that the number of B lymphocytes in the bone marrow, lymph nodes and Peyer's patches of these mice was significantly higher than in the same organs of sham-operated mice (Rozing *et al.*, 1977a). These results were interpreted as indicating the existence of a regulating compensatory mechanism at the B lymphocyte level. Nevertheless, in these mice a significantly lower concentration of serum IgM was found (Rozing *et al.*, 1977a). Besides these phenomena caused by adult splenectomy, neonatal splenectomy and congenital absence of the spleen in mice have been claimed to affect the ability of bone marrow and thymus cells to co-operate in the adoptive immune response against sheep red blood cells (SRBC) (Bucsi, Borek & Battisto, 1972; Wargon, Lozzio & Wust, 1975).

In the present paper the defect in the immune system responsible for the consistently low IgM concentration in the blood after splenectomy has been investigated and the synergistic capacity of bone marrow and thymus cells of neonatally splenectomized mice in the adoptive plaque-forming cell (PFC) response against SRBC has been analysed.

MATERIALS AND METHODS

Animals

(C57Bl/Rij × CBA/Rij) F1 female mice were used. They were purchased from the Medical Biological Laboratory, Rijswijk, The Netherlands and from The Laboratory Animals Centre of the Erasmus University, Rotterdam, The Netherlands.

Splenectomy

Adult splenectomy and sham-splenectomy were performed on mice at 14–20 weeks of age as described previously (Rozing *et al.*, 1977a). Mice were anaesthetized with Nembutal (Abbott S.A., Saint-Rémy-sur-Avre, France) (70 mg/kg body weight). There was no post-operative mortality.

Neonatal splenectomy and sham-splenectomy were performed on new-born mice within 24 h after birth using the technique of Haller (1964). Mice were anaesthetized by placing them on ice. If bleeding occurred during the splenectomy procedure, such mice were excluded from experiments, since bleeding resulted in the formation of spleen-nodules with a normal splenic histology (Rozing, unpublished observation). The abdomen of the splenectomized mice was carefully inspected for splenic remnants. There was less than 10% post-operative mortality.

Cell suspensions

Cell suspensions of spleen, bone marrow, and lymph nodes (inguinal, brachial, axillary and mesenteric) were prepared in a balanced salt solution as described previously (Rozing *et al.*, 1977b). Nucleated cells were counted with a Coulter Counter Model B. Results obtained from the femoral marrow were extrapolated to the marrow present in the whole animal using the data of Chervenick, Boggs, Marsh, Cartwright & Wintrobe (1968), who showed that in mice one femur contains 5·9% of the total bone marrow. Corticosteroid resistant thymocytes (CRT) were obtained from 6-week-old mice injected i.p. with 30 mg of the synthetic corticosteroid Dexamethasone sodium phosphate (Merck & Co., Rahway, New Jersey) per kg body weight 2 days previously.

Immunofluorescence staining of B cells

Before reacting with the conjugate, the cells were washed three times in a solution consisting of 5% bovine albumin in phosphate-buffered saline (5% BA-PBS). For detection of B lymphocytes a rhodamine-conjugated goat anti-mouse immunoglobulin serum (TRITC-GaM-Ig) (Nordic, Tilburg, The Netherlands) was used in a direct vital staining technique. Details of the staining technique and the specificity of the TRITC-GaM-Ig have been described elsewhere (Rozing *et al.*, 1976). For detection of IgM-carrying B lymphocytes an identical procedure was used with a fluorescein conjugated specific rabbit anti-mouse-IgM serum (FITC-RaM-IgM). The FITC-RaM-IgM was a gift of Dr J. J. Haaijman and Dr J. Rádl from the Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands. The TRITC-GaM-Ig reacted with all classes of mouse immunoglobulins, whereas the FITC-RaM-IgM reacted only with IgM.

Slides were examined with a Zeiss fluorescence microscope equipped with a vertical illuminator IV/F and an Osram HBO 50 mercury lamp. Cells with the morphology of lymphocytes displaying a ring, a cap or speckled peripheral fluorescence were scored as positive. Dead cells, which occurred occasionally, showing a uniform fluorescence, were not scored.

X-irradiation

Recipient mice received 925 rad whole body X-irradiation, generated in a Philips Mueller MG 300 X-ray machine, as described in detail previously (Rozing *et al.*, 1976). Irradiated control mice died in 9–13 days.

Adoptive PFC response

Irradiated recipients were i.v. injected with the appropriate cell suspension and 4×10^8 SRBC within 4 h after irradiation. Each group consisted of 5 mice. On the fourth day all mice were boosted with 4×10^8 SRBC i.p. The spleen of the recipients was removed on day 7 and the number of direct (IgM) plaque-forming cells (PFC) and indirect (IgG) PFC was determined in the plaque assay as described in detail elsewhere (Rozing *et al.*, 1976). The 95% confidence limits associated with the number of plaques counted was calculated as described previously. The presence of IgG-PFC was accepted as significant when there was no overlap between the upper limit in the direct assay and the lower limit in the indirect assay.

Immunofluorescence staining of plasma cells

After washing the cells three times in 5% BA-PBS, cytocentrifuge slides were made using a Shandon Elliott Cytocentrifuge. Before reacting with the conjugate the cells were fixed for 20 min in cold ethanol-acetic acid (−20°) according to the procedure as described by Haaijman, Schuit & Hijmans (1977). For detection of Ig-containing and IgM-containing plasma cells the same antisera were used as mentioned under B cell staining. Plasma cells were stained and quantified as described by Haaijmans *et al.* (1977).

Serum immunoglobulins

Serum immunoglobulin concentrations (IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgA) were determined using radial immunodiffusion plates obtained from Meloy Laboratories, Inc., Springfield, U.S.A.

RESULTS

Immunoglobulin positive (B) lymphocytes

The number of B lymphocytes was determined in the bone marrow and lymph nodes of both adult and neonatally splenectomized mice and in the spleen, bone marrow and lymph nodes of sham-operated animals 3–4 months after surgery. To estimate the total number of B cells we used a membrane-fluorescence technique with a specific anti-immunoglobulin conjugate as reagent, while a specific

Table 1. Number of Ig-positive (B) lymphocytes in various organs of neonatally and adult splenectomized or sham-splenectomized mice

Organ		ShSx*	Sx*	nShSx†	nSx†
Spleen	Percent‡	43·0 ± 2·9¶	—	47·8 ± 2·5	—
	Number§	6·9 ± 0·4	—	7·5 ± 0·4	—
Bone marrow	Percent	9·8 ± 1·2	15·5 ± 0·7	10·7 ± 2·0	16·1 ± 0·4
	Number	4·7 ± 0·6	6·8 ± 0·3	4·6 ± 0·9	8·0 ± 0·2
Lymph nodes	Percent	23·1 ± 1·2	34·1 ± 2·8	23·5 ± 1·9	31·2 ± 2·2
	Number	0·3 ± 0·1	0·6 ± 0·1	0·3 ± 0·1	0·7 ± 0·1

* Mice were splenectomized (Sx) or sham-splenectomized (ShSx) between 14–20 weeks of age.

† Mice were neonatally splenectomized (nSx) or sham-splenectomized (nShSx) within 24 h after birth.

‡ Percentage of B cells is expressed as percentage of all nucleated cells.

§ Number of B cells ($\times 10^{-7}$) is obtained by combining total cell numbers (data not shown) and percentages B cells as expressed under ‡.

¶ Average ± 1 s.e.m. Each group consisted of at least 5 mice.

Table 2. Number of IgM-positive (B) lymphocytes in various organs of neonatally and adult splenectomized or sham-splenectomized mice

Organ			ShSx*	Sx*	nShSx†	nSx†
Spleen	Percent	IgM-B cells‡	35·0 ± 2·7¶	—	39·4 ± 1·3	—
	Ratio	$\frac{\text{IgM-B cells}}{\text{Ig-B cells}}$§	0·81 ± 0·01	—	0·83 ± 0·02	—
Bone marrow	Percent	IgM-B cells	9·0 ± 0·9	14·7 ± 0·8	9·8 ± 1·6	14·8 ± 0·6
	Ratio	$\frac{\text{IgM-B cells}}{\text{Ig-B cells}}$	0·92 ± 0·03	0·95 ± 0·03	0·93 ± 0·03	0·92 ± 0·02
Lymph nodes	Percent	IgM-B cells	19·9 ± 1·7	29·5 ± 2·0	19·2 ± 1·5	26·1 ± 2·0
	Ratio	$\frac{\text{IgM-B cells}}{\text{Ig-B cells}}$	0·87 ± 0·03	0·86 ± 0·02	0·82 ± 0·03	0·83 ± 0·01

* Mice were splenectomized (Sx) or sham-splenectomized (ShSx) between 14–20 weeks of age.

† Mice were neonatally splenectomized (nSx) or sham-splenectomized (nShSx) within 24 h after birth.

‡ Percentage IgM-positive B cells is expressed as percentage of all nucleated cells.

§ Ratio between IgM-positive B cells and all B cells is obtained by dividing the number of IgM-B cells by the total number of B cells.

¶ Average ± 1 s.e.m. Each group consisted of at least 5 mice.

anti-IgM conjugate was used for the determination of IgM-positive B cells.

By comparing the number of B cells in the various organs of splenectomized and sham-splenectomized mice we found that in the bone marrow and lymph nodes of splenectomized mice the number of B lymphocytes was significantly higher (Table 1). The increased number of B lymphocytes in splenectomized mice was caused by a specific enlargement of the B-cell population in the various organs and not by a non-specific growth of the total cellularity of these organs, as can be concluded from the increased percentages of B cells. Also a significantly higher percentage of IgM-positive B lymphocytes was found in the bone marrow and lymph nodes of splenectomized mice than in the same organs of sham-splenectomized mice (Table 2). From the fact that the ratio between IgM-B cells and total B cells in the various organs is not influenced by splenectomy (Table 2), it can be concluded that the predominance of IgM-carrying B lymphocytes remains after splenectomy and that the increase of the total number of B lymphocytes and the number of IgM-B lymphocytes is of about the same magnitude in the various organs.

Adoptive PFC response to SRBC

To test the ability of B lymphocytes of splenectomized mice to differentiate into antibody forming cells, 2×10^7 bone marrow cells of these mice were transferred into irradiated recipients in combination with 10^7 corticosteroid resistant thymocytes (CRT) and 4×10^8 SRBC. The CRT were added to avoid a limitation of the immune response by a lack of helper T-cells. A dose of 10^7 CRT has been shown to be an optimal dose of helper T-cells in this system (Van Muiswinkel, Zaalberg, Majoor, Lubbe, Van Soest & Van Beek, 1975). Using this cell transfer system, we found that in both groups of splenectomized mice the B lymphocytes in the bone marrow were fully capable to differentiate into IgM- and IgG-producing cells. Bone marrow cells from splenectomized mice gave rise to about twice as many IgM-PFC and IgG-PFC upon adoptive transfer into irradiated recipients, as did the same number of bone marrow cells from sham-splenectomized mice (Table 3). This difference is of about the same magnitude as found for the total number of B cells (Table 1) and the number of IgM-B cells (Table 2), comparing the bone marrow of splenectomized and sham-splenectomized mice.

Immunoglobulin containing cells

Immunoglobulin containing (C-Ig) cells were determined in bone marrow and lymph nodes of splenectomized mice and in spleen, bone marrow and lymph nodes of sham-splenectomized mice. As shown in Table 4 the majority of plasma cells in

Table 3. Adoptive PFC response to SRBC of bone marrow cells from neonatally and adult splenectomized or sham-splenectomized mice

Experimental group*	IgM-PFC/spleen†	IgG-PFC/spleen†
Sx‡	17,410¶ (14,117 – 21,470)	59,866 (42,906 – 83,530)
ShSx‡	8397 (7466 – 9443)	25,466 (16,844 – 38,500)
nSx§	32,469 (28,002 – 37,648)	55,198 (40,845 – 74,593)
nShSx§	14,678 (13,520 – 15,935)	30,680 (19,808 – 47,520)

* 2×10^7 Bone marrow cells were transferred into irradiated recipients in combination with 10^7 CRT and 4×10^8 SRBC.

† Direct (IgM) and indirect (IgG) PFC assay was done on the spleen of the recipient mice 7 days after cell transfer.

‡ Mice were splenectomized (Sx) or sham-splenectomized (ShSx) between 14–20 weeks of age.

§ Mice were neonatally splenectomized (nSx) or sham-splenectomized (nShSx) within 24 h after birth.

¶ Geometric mean and 95% confidence limits. Each group consisted of at least 5 mice.

Table 4. Number of Ig-containing cells in various organs of adult splenectomized or sham-splenectomized mice

	Experimental group*	Total C-Ig cells† $\times 10^{-3}$	% C-IgM cells‡	Total C-IgM cells† $\times 10^{-3}$
Spleen	ShSx	310 ± 35§	77 ± 6	239 ± 30
	Sx	—	—	—
Bone marrow	ShSx	313 ± 48	22 ± 8	70 ± 23
	Sx	457 ± 62	30 ± 5	135 ± 28
Lymph nodes	ShSx	38 ± 12	8 ± 4	3 ± 2
	Sx	63 ± 18	11 ± 7	7 ± 6
Total¶	ShSx	661		312
	Sx	520		142

* Mice were splenectomized (Sx) or sham-splenectomized (ShSx) between 14–20 weeks of age.

† Numbers of Ig containing cells (C-Ig) and IgM containing cells (C-IgM) are expressed per organ.

‡ Percentage IgM containing cells (C-IgM) is expressed as a percentage of all Ig containing cells (C-Ig).

§ Average ± 1 s.e.m. Each group consisted of at least 6 mice.

¶ Total numbers of C-Ig cells and C-IgM cells summed over the three indicated organs.

sham-operated mice was located in the spleen and in the bone marrow, whereas a minor proportion of plasma cells occurred in the lymph nodes. Obviously IgM-containing (C-IgM) cells were mainly located in the spleen. After splenectomy the number of C-Ig cells in the bone marrow and lymph nodes of splenectomized mice increased as compared with sham-operated animals. Also the number of C-IgM cells was higher in the organs of splenectomized mice. However, although the higher number of C-Ig cells in bone marrow and lymph nodes of splenectomized mice resulted in almost normal total numbers of C-Ig cells, the total number of C-IgM cells in splenectomized mice was only 50 per cent of the number of C-IgM cells in sham-splenectomized mice.

Serum immunoglobulin concentration

In order to investigate the influence of splenectomy on the serum-immunoglobulin levels the concentrations of IgM, IgG_1, IgG_{2a}, IgG_{2b} and IgA were determined in the blood of neonatally and adult splenectomized and sham-splenectomized mice. No significant differences were found between splenectomized and sham-splenectomized mice for IgG_1, IgG_{2a}, IgG_{2b} and IgA (Table 5). The only clear

Table 5. Immunoglobulin concentrations in the blood of neonatally and adult splenectomized or sham-splenectomized mice

Experimental group	Immunoglobulin class					
	IgG_1	IgG_{2a}	IgG_{2b}	IgA	IgM	Total*
Sx†	2·13 ± 0·44§	1·76 ± 0·11	1·18 ± 0·05	1·37 ± 0·16	0·32 ± 0·03	6·76 ± 0·56
ShSx†	1·28 ± 0·13	1·86 ± 0·11	1·11 ± 0·07	0·91 ± 0·14	0·56 ± 0·03	5·72 ± 0·32
nSx‡	2·08 ± 0·34	2·10 ± 0·18	1·11 ± 0·13	2·71 ± 0·46	0·29 ± 0·04	8·29 ± 0·92
nShSx‡	2·24 ± 0·35	1·60 ± 0·07	0·87 ± 0·07	2·11 ± 0·54	0·60 ± 0·07	7·42 ± 0·67

* Total immunoglobulin concentration is obtained by summing the data of the various classes.
† Mice were splenectomized (Sx) or sham-splenectomized (ShSx) between 14–20 weeks of age.
‡ Mice were neonatally splenectomized (nSx) or sham-splenectomized (nShSx) within 24 h after birth.
§ Immunoglobulin concentrations (± 1 s.e.m.) in mg protein per ml. Each group consisted of at least 10 mice.

effect of both adult and neonatal splenectomy on serum immunoglobulin concentrations occurred in the IgM class. As has been found in humans (Zierott, Zenker & Thiede, 1973; Claret *et al.*, 1975), splenectomy of mice caused a significant decrease of the IgM concentration in the blood. Total serum immunoglobulin concentrations proved to be the same in splenectomized and sham-splenectomized mice.

Bone marrow-thymus synergism

Neonatal splenectomy and congenital absence of the spleen have been reported to lead to a decreased capacity of bone marrow and thymus cells to co-operate in the adoptive PFC response to SRBC (Bucsi *et al.*, 1972; Wargon *et al.*, 1975). However, in previous experiments in our laboratory with splenectomized, irradiated and foetal liver reconstituted mice (Rozing *et al.*, 1977a) no such failure could be demonstrated for bone marrow and thymus cells of these mice. Since there is an analogy between the circumstances under which lymphocytes develop in neonatally splenectomized mice and in splenectomized irradiated and reconstituted mice, we investigated in the present experiments the syner-

Table 6. Ability of bone marrow cells and thymocytes of neonatally splenectomized or sham-splenectomized mice to co-operate in the adoptive PFC response to SRBC

Experimental group*	IgM-PFC/spleen†	IgG-PFC/spleen†
BMnSx	285‡ (154 – 432)	85 (n.s.)§
BMnSx + THnSx	2084 (1673 – 2596)	718 (n.s.)
BMnSx + THnShSx	2539 (1914 – 3368)	195 (n.s.)
BMnShSx	736 (578 – 937)	400 (n.s.)
BMnShSx + THnSx	7707 (5900 – 10,067)	5771 (3164 – 10,525)
BMnShSx + THnShSx	10,489 (8725 – 12,610)	319 (n.s.)

* 2×10^7 Bone marrow cells (BM) together with or without 2×10^7 thymocytes (TH) of mice splenectomized (nSx) or sham-splenectomized (nShSx) within 24 h after birth were transferred into irradiated recipients together with 4×10^8 SRBC.
† Direct (IgM) and indirect (IgG) PFC-assay was done on the spleen of the recipient mice 7 days after cell transfer.
‡ Geometric mean and 95% confidence limits. Each group consisted of at least 5 mice.
§ n.s. means that the number of PFC found in the indirect PFC-assay did not differ significantly from the number of PFC found in the direct PFC-assay.

gistic ability of bone marrow and thymus cells of neonatally splenectomized mice in the adoptive PFC response to SRBC. Table 6 shows that bone marrow cells of splenectomized mice transferred in combination with thymocytes from the same mice evoked an equal increase of the number of PFC against SRBC as did these marrow cells transferred with thymocytes from sham-splenectomized mice, as compared with the transfer of such marrow cells alone. The same pattern occurred upon the transfer of bone marrow cells of sham-splenectomized mice in combination with or without thymocytes from splenectomized and sham-splenectomized mice. Since the transfer of bone marrow cells of neonatally splenectomized mice together with cortisone resistant thymocytes as helper T-cells and SRBC resulted in an excellent adoptive PFC response to SRBC (Table 3), it can be concluded from these experiments that bone marrow and thymus cells of neonatally splenectomized mice are fully capable to co-operate in the immune response to SRBC. The seemingly contradictory observation in Table 6 (cf. Table 3) that bone marrow cells of neonatally sham-splenectomized mice evoked slightly higher adoptive PFC responses than bone marrow cells of neonatally splenectomized mice did, is probably the result of a sub-optimal test system using bone marrow cells alone or in combination with normal thymocytes as helper T-cells (Van Muiswinkel *et al.*, 1975).

DISCUSSION

The spleen seems to be an important organ for the differentiation and maturation of haemopoietic stem cells to immunocompetent B lymphocytes. In normal mice the bone marrow is probably the major source of B lymphocytes (Osmond, 1975), while the spleen is supposed to play an important role in the subsequent maturation of B cells (Strober, 1976). Basten, Miller, Warner & Pye (1971) were able to inactivate splenic B lymphocytes using a radioactively-labelled antigen, whereas bone marrow lymphocytes under identical conditions were unaffected. Furthermore, it was found by Stocker, Osmond & Nossal (1974) that per Ig-positive B lymphocyte the adoptive PFC response of spleen cells to the T-cell-independent antigen dinitrophenylated polymer of flagellin (DNP-POL) is three times higher than the response of bone marrow cells. The difference in the length of time required to induce tolerance in bone marrow and splenic B cells, reported by Chiller & Weigle (1973), may also be related to differences in antigen-binding capacities of the two cell populations, since the first step of tolerance induction is the binding of tolerogen to immunoglobulin receptors. Indeed Osmond & Nossal (1974) found that the mean density of immunoglobulin receptors on splenic B-lymphocytes is higher than on bone marrow B-cells.

However, from the data presented in this paper and from experiments using splenectomized, irradiated and foetal liver reconstituted mice (Rozing *et al.*, 1977a) it is obvious, that this maturation process can also occur outside the spleen. B lymphocytes in the bone marrow of splenectomized mice were found to be fully immunocompetent, since the increase in number of Ig-positive B cells (Table 1) was related to a rise of the adoptive PFC response of bone marrow cells after transfer into irradiated recipients of the same magnitude (Table 3). Obviously, in splenectomized mice compensation occurred not only for the absence of the splenic B-cell population by an increase of the number of B lymphocytes in the remaining organs, but also the role of the spleen in the maturation process of B lymphocytes must have been taken over by the other organs. Since also the total number of immunoglobulin-producing plasma cells in splenectomized mice was about the same as in sham-splenectomized mice (Table 4), corresponding with a normal serum immunoglobulin concentration in splenectomized mice (Table 5), this compensatory mechanism proved to result in an almost normal function of the humoral immune system.

In contrast to total serum immunoglobulins, the IgM concentration in the blood of splenectomized mice was significantly reduced as compared to sham-operated mice (Table 5). Such a decrease has also been found in patients after splenectomy (Zierott *et al.*, 1973; Claret *et al.*, 1975). The majority of IgM-synthetizing plasma cells in normal mice is located in the spleen (Haaijman *et al.*, 1977). The low concentration of IgM in the blood of splenectomized mice was caused by a failure of the remaining organs to compensate completely for the absence of splenic IgM-producing plasma cells. Since in the bone marrow and lymph nodes the numbers of precursors of such plasma cells, the IgM-positive B lymphocytes, and their ability to differentiate into IgM-synthetizing PFC was not affected by splenec-

tomy, it suggests that the differentiation from IgM-positive B lymphocytes into IgM-producing plasma cells is defective in these tissues and they provide a less efficient environment for this differentiation step than the spleen. Patients splenectomized for various reasons fail to produce circulating IgM-antibodies upon intravenous injection of heterologous erythrocytes (Rowley, 1950). Similar results were obtained in splenectomized rats and dogs by Van Wijck, Witte, Witte & Strunk (1976) in mice by Benner & Van Oudenaren (1975) and in the primary response after intravenous injection with sheep erythrocytes. Probably as a consequence of the reduced IgM-production, splenectomized individuals have a greater risk to develop fulminant and fatal sepsis due to normally non-fatal bacterial infections, such as pneumococcus and meningococcus infections (Singer, 1973). Non-surgical asplenic states such as congenital absence of the spleen (Kevy, Teft, Vawter & Rosen, 1968) and autosplenectomy in sickle-cell disease syndromes (Barrett-Connor, 1971) also have increased risk of serious bacterial infection. Shinefield and colleagues (Shinefield, Steinberg & Kaye, 1966) reported that the susceptibility to infections with *Diplococcus pneumoniae* was increased in splenectomized mice. These bacteria do not have potent endotoxins (Macleod, 1965) and the defence against these micro-organisms greatly depends on the coating with opsonizing antibodies, which are predominantly of the IgM-class (Spiegelberg, 1974). The role of the spleen in the defence against such bacterial infections is a double one, i.e. it provides a site of production of specific antibodies as well as a filter system in the blood stream, which permits phagocytosis of bacteria by the cells of the reticulo-endothelial system. Possibly the main effect of splenectomy in the development of fatal sepsis results from the loss of splenic phagocytic capacity. However, in an elegant set of experiments Haller (1970) showed that in the defence against *D. pneumoniae* the production of opsonizing antibodies in the spleen was more important than the phagocytic capacity of this organ.

From *in vitro* studies on the interdependence of lymphoid organs during ontogeny performed by Auerbach (1963) it can be concluded that there exists during embryological life a splenic influence stimulating the proliferation and differentiation of thymic cells. To study whether *in vivo* a splenic influence on B and/or T lymphocyte maturation in bone marrow and thymus exists during neonatal life, we analysed the role of the spleen in the development of immune competence during that period. We examined the synergistic ability of bone marrow and thymus cells of neonatally splenectomized mice. The functional capacities of both of these cell populations was normal. Therefore there is no critical regulatory influence of the spleen *in vivo* upon lymphocyte differentiation and maturation during neonatal life. These results are in agreement with those reported by Auerbach and coworkers (1976) in neonatally splenectomized mice and with our previous experiments on the co-operation between bone marrow and thymus cells of splenectomized, irradiated and reconstituted mice in the adoptive PFC response to SRBC (Rozing *et al.*, 1977a). Nevertheless Bucsi *et al.* (1972) and Wargon *et al.* (1975) presented evidence, that bone marrow cells and thymocytes of congenitally spleenless or neonatally splenectomized mice were defective in their capacity to co-operate. These contradictory observations are probably attributable to differences in sensitivity of the test system used, as suggested by Auerbach and colleagues (1976).

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