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ROLE OF SPLEEN IN ENDOTOXIN POISONING AND RETICULOENDOTHELIAL FUNCTION

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Summary.—The nature of cellular factors in host response to endotoxin was determined by studying various phases of reactivity to endotoxin in splenectomized and sham operated mice. Spleen ablation by itself actually increased the resistance of animals to endotoxin lethality and the absence of spleen did not interfere with either the development or expression of tolerance to the lethal effects of the toxin. The clearance rates of carbon and of endotoxin were normal after splenectomy and increased in conjunction with RES activation associated with endotoxin tolerance, both in sham operated and the splenectomized animals. From these results, it is concluded that subtle and specific adaptations of hepatic RE elements and/or critical metabolic functions play a determining role in endotoxicosis. Furthermore, the presence of spleen does not seem to be required for opsonization, phagocytosis and removal of either nonspecific, inert colloidal particles such as carbon, or of biological materials such as endotoxin. Pertinence of these findings to splenectomized patients is indicated.

AT the present time much controversy exists with respect to the critical mechanism(s) that determines the subsequent outcome of events in host response to endotoxins. It has been reported that humoral detoxification plays a paramount role in endotoxin poisoning (Skarnes, 1966, 1968). Comparison of the host blood volume and the ability to withstand endotoxic shock among various species, however, makes it extremely unlikely that blood detoxification would play an important role in endotoxicosis. Thus it became relevant to assess the contribution of the liver relative to that of the spleen since these 2 organs together trap almost all of the intravenously administered endotoxin (Chedid *et al.*, 1964, 1971). Some authors believe that the ability of the spleen to detoxify endotoxin represents the major resistant factor in endointoxicated animals (Rutenberg *et al.*, 1965, 1967). Our previous studies, on the other hand, had shown that all phases of endotoxin poisoning could be more readily explained as subtle and specific changes in liver function (Agarwal, 1972). This problem was directly and conclusively assessed in splenectomized mice and the results are reported here.

MATERIALS AND METHODS

Male, Swiss mice (24 $\pm$ 2 g body weight) were used in all experiments. Animals were housed on vermiculite bedding; food and water were available at all times.

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Splenectomy or sham operation was performed via retroperitoneal approach under ether anaesthesia and animals were used 24–48 hours after surgery. *Escherichia coli* endotoxin (Difco) was used in doses proportional to LD_{50} (*Ca.* 250 μg for a mouse of 25 g). Survival was recorded 48 hours after challenge in all cases.

The carbon clearance test of Benacerraf and Sebestyen (1957) was used to determine the phagocytic activity of the RES exactly as previously described (Agarwal, 1972). To determine intravascular clearance, *Salmonella enteritidis* (Danysz) endotoxin was labelled with $Na_2\ ^{51}Cr\ O_4$ and employed as described in detail on previous occasions (Chedid *et al.*, 1964, 1971).

Isotonic, non-pyrogenic saline (Meram Laboratories, Paris) was used as a diluent for endotoxin and the carbon suspension (C11/1431a; Gunther–Wagner, Hanover, Germany). Intraperitoneal injections were made in a volume of 0·5 ml and intravenous in 0·2 ml. The carbon suspension and the radioactive endotoxin were administered intravenously; all other injections were given intraperitoneally.

RESULTS

Effect of splenectomy on endotoxin lethality

Data in Table I show conclusively that the LD_{50} for endotoxin (565 μg) in the sham operated mice was effectively elevated over the control level in animals whose spleen had been removed 24 hours before challenge. Thus, spleen ablation actually increased the ability of mice to withstand lethal endotoxic shock.

Repeated injections of endotoxin in a normal animal are known to induce a state of tolerance to various obnoxious effects of the toxin (Beeson, 1947; Bennett

TABLE I.—*Effect of Splenectomy on Various Phases of Reactivity to Endotoxin in Mice*

Treatment before test*	Challenging dose (μg)	Dead/Total	LD_{50} Post-challenge (μg)
1. Sham operated (normal)	100	0/8	565
	200	0/8	
	400	1/8	
	800	7/8	
2. Splenectomized (normal)	100	0/8	> 800
	200	0/8	
	400	0/8	
	800	2/8	
3. Tolerant (sham operated Day 0)	400	0/8	1130
	800	2/8	
	1600	6/8	
	3200	8/8	
4. Tolerant (splenectomized Day 0)	400	0/8	1310
	800	1/8	
	1600	5/8	
	3200	8/8	
5. Sham operated (tolerant)	400	1/8	1210
	800	0/8	
	1600	6/8	
	3200	8/8	
6. Splenectomized (tolerant)	400	0/8	1270
	800	2/8	
	1600	4/8	
	3200	8/8	

* For details see text.

and Cluff, 1957). It is not known whether spleen contributes to establishing the state of tolerance to endotoxin. Animals were sham operated or splenectomized on Day 1 and injected with progressively increasing doses of endotoxin (5, 5, 10, 10 and 20 μg) on successive days, until Day 6. The LD_{50} of endotoxin in both groups, challenged 24 hours after the last injection, was elevated to twice the level seen in sham operated, non-tolerant animals (compare lines 1, 3 and 4, Table I). Thus, absence of spleen did not apparently modify the development of tolerance to endotoxin lethality.

The possibility exists that these results in splenectomized mice may not represent the usual situation where the presence of spleen during induction of tolerance to endotoxin might require an enlarged spleen for subsequent expression of the tolerant state. To this end, normal mice were given repeated injections of endotoxin (as above), sham operated or splenectomized 24 hours after the last injection and then challenged 24 hours after operation. At the time of surgery, animals so treated exhibited marked splenomegaly (as is usually associated with the tolerant state). Data in the last part of Table I show that the LD_{50} of endotoxin was nearly identical both in tolerant-sham operated and tolerant-splenectomized animals. Furthermore, this LD_{50} of endotoxin was no different from that in mice whose spleen had been removed before induction of tolerance.

Thus, the results shown in this Table clearly establish that the presence of spleen actually reduces the ability of mice to withstand lethal endotoxic shock and that spleen ablation does not alter either the establishment or the expression of tolerance to endotoxin lethality.

Effect of splenectomy on reticuloendothelial function

Attention was next directed to the contribution of the reticuloendothelial system (RES) during various phases of reactivity to endotoxin in splenectomized mice, since the RES is believed to play a determining role in endotoxicosis (Beeson, 1947).

TABLE II.—*Lack of Effect of Splenectomy on RES Activity in Mice*

Treatment before test*	K value	Body (g)	Liver (mg)	Spleen (mg)
Sham	0·029±0·003	29·4±0·5	1611±76	193±30
Splenectomized	0·025±0·002	31·0±0·4	1563±73	—

* Animals were tested 24 hours after sham operation or splenectomy. Each value is the average of 7–8 individual determinations ± standard error.

Data in Table II show that 24 hours after splenectomy the carbon clearance rate, the body and the liver weights were significantly the same as those of sham operated mice. In other experiments it was found that these values were similar to those seen in normal mice without surgical trauma. Thus, the increased ability of splenectomized mice to withstand lethal effects of endotoxin could not be related to a non-specific or general activation of the RES.

A similar type of determination was made in mice in which tolerance to endotoxin was established after removal of the spleen. Data in Table III show that the activation of the RES, associated with endotoxin tolerance, progressed normally in both sham operated and splenectomized mice. This clearly establishes that spleen contributes little, if any, to the increased rate of carbon clearance

TABLE III.—*Evidence that Spleen is Not Necessary for RES Activation Associated with Endotoxin Tolerance*

Treatment before test*	K value	Body (g)	Liver (mg)	Spleen (mg)
Sham	0·068±0·005	25·9±0·8	1626±76	280±15
Splenectomized	0·063±0·004	24·8±0·5	1630±76	—

* Twenty-four hours after splenectomy or sham operation, animals were given endotoxin for 5 consecutive days and tested 24 hours after the last injection. Each value is the average of 7–8 individual determinations ± standard error.

that develops with endotoxin tolerance. If prior opsonization of carbon is required for subsequent efficient removal, it is evident therefore that such factors either do not become limiting under these conditions or that spleen is not necessary for elicitation or expression of such components.

TABLE IV.—*Unaltered Expression of RES Activation Following Splenectomy in Endotoxin Tolerant Mice*

Treatment before test*	K value	Body (g)	Liver (mg)	Spleen (mg)
Sham	0·052±0·006	25·8±0·9	1608±93	232±18
Splenectomized	0·059±0·008	25·6±0·9	1675±79	—

* Mice were given endotoxin for 5 consecutive days. Splenectomy or sham operation was performed 24 hours after the last injection and animals were tested 24 hours thereafter. Each value is the average of 7–8 individual determinations ± standard error.

These relationships become further evident by perusal of data in Table IV. When spleen was removed in mice that had previously been rendered tolerant to endotoxin, the K or liver/body weight ratios were no different from tolerant, sham operated animals. Furthermore, the magnitude of changes was similar to that observed for groups shown in Table III. Thus, the pathways leading to RE activation concurrently with endotoxin tolerance were equally operative both in the presence and the absence of spleen. It is established, therefore, that the presence of spleen is not required either for normal carbon clearance rate or for its activation or expression associated with endotoxin tolerance.

Effect of splenectomy on clearance of endotoxin

In view of the fact that splenectomy did not influence any phase of carbon clearance, it became of interest to see whether spleen contributes to clearance of endotoxin. This is important because endotoxin and carbon clearance can be dissociated from one another (Agarwal, 1972), and because opsonins may be required for clearance of a biological material like the endotoxin (Rowley, 1962).

Data in Fig. 1 show that splenectomy did not influence clearance of endotoxin at any of the 3 time points studied. This demonstrates that if the presence of spleen is required for elicitation of factors necessary for removal of circulating endotoxin from the blood, these do not become limiting 24 hours after spleen ablation.

Data in Table V reveal that endotoxin clearance was elevated in sham operated mice rendered tolerant by repeated injections of the toxin. It is clear that the rates of removal of humorally introduced endotoxin were equally great in mice

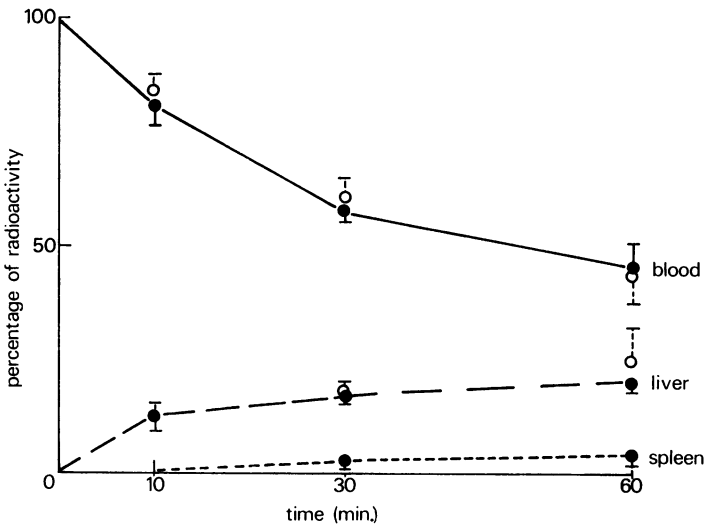

FIG.—Unaltered endotoxin clearance rates following splenectomy.
Twenty-four hours after splenectomy (○) or sham operation (●) mice were injected with 10 μg of *S. enteritidis* endotoxin (0·04 LD_{50}), containing approximately 15,000 ct/min intravenously. The percentage of the injected radioactivity in all compartments was determined individually on 5 separate animals at each of the indicated time points. Standard error on the mean average in each group indicates the experimental variation.

TABLE V.—*Lack of Effect of Splenectomy on Endotoxin Clearance in Tolerant Mice*

Treatment before test*	Time	Percentage of radioactivity	
		Blood	Liver
Control (non-tolerant)	3 minutes	81·0±1·2	7·2±1·1
	10 minutes	74·0±3·0	11·7±0·4
Sham operated (before tolerance)	3 minutes	64·1±4·0	16·3±4·9
	10 minutes	40·9±11·5	37·8±9·7
Splenectomized (before tolerance)	3 minutes	69·5±2·3	13·4±0·7
	10 minutes	48·0±5·0	32·2±5·8

* Animals were sham operated or splenectomized and injected with progressively larger amounts of endotoxin for 5 consecutive days and used 24 hours after the last injection. Controls were given only saline. For details of clearance see legend to Fig.

whose spleen had been removed before the induction of tolerance. Thus, ablation of spleen 7 days beforehand did not influence activation of factors that may be required for subsequent efficient removal of endotoxin.

DISCUSSION

The results reported here establish clearly that the ability of the spleen to detoxify endotoxin has little to do with resistance of mice to endotoxin lethality. Indeed, spleen ablation actually increased the ability of animals to withstand challenge with a lethal dose of the toxin. This may be interpreted to mean that

the presence of spleen results in elicitation of certain factors that might influence a more primary sensitive site, thereby lowering the resistance of the animal to endotoxin death. Granuloma formation in the spleen is postulated to be responsible for the unusually high sensitivity of BCG infected mice to withstand endotoxic shock, and splenectomy in such animals actually increases resistance to endotoxin lethality (Chedid *et al.*, 1971). It remains to be seen whether a similar mechanism could explain the results reported here. Neither the development nor the expression of endotoxin tolerance, nor the activation of the RES associated with the tolerant state, could be influenced by the absence of spleen. Thus it is evident that answers to the problem of endotoxicosis must be found in a target other than the spleen. It should be pointed out that sham operated tolerant mice possessed enlarged spleens, yet they were no more resistant to the lethal effects of endotoxin than splenectomized tolerant animals. Because liver traps most of the intravenously injected endotoxin, this organ appears to play an obvious role in endotoxaemia.

Earlier it had been indicated that blood detoxification mechanisms do not alone suffice to explain the various facets of endotoxin poisoning. Nevertheless, it was conceivable that efficient removal from the blood stream might be a prerequisite for eventual cellular handling.

We have previously shown that carbon clearance could be dissociated from clearance of endotoxin (Agarwal, 1972). Results described here reveal that the presence of spleen is not required for the activation and expression of augmented rates of clearance of either carbon or endotoxin that are usually associated with the tolerant state. Splenectomy, in addition, increased resistance to endotoxin without lowering the innate pattern of endotoxin clearance. This further indicates that gross changes in clearance and/or distribution patterns cannot alone account for the various phases of reactivity to endotoxin observed after lowering or increasing the resistance of an animal to endotoxin lethality (Noyes, McInturf and Blahuta, 1959). Therefore, a causal contribution of humoral compartment in host response to endotoxin seems dubious. It cannot be said at the present time whether splenectomy activates accessory sites for elicitation of any humoral components that might be necessary for augmented clearance of intravascularly injected substances. We must also consider the possibility that spleen may be causally related to elicitation of humoral factors that can passively elevate resistance against endotoxin lethality in a normal recipient (Agarwal and Berry, 1968; Freedman, 1959).

It follows then that the nature of the changes in the liver of animals actively injected with endotoxin could involve specific and subtle adaptations of the RE elements (to process the phagocytozed antigen) or selected modifications in certain critical metabolic functions, or a complementation of both these tenets. These aspects have been discussed in detail in a recent report from this laboratory, showing that all phases of reactivity to endotoxin could be readily explained as appropriate changes in liver function (Agarwal, 1972). It remains to be seen whether splenectomy would alter the increase in non-specific resistance to various types of infection that follows endotoxin administration. Further studies are required to delineate the nature of adaptations in the liver and to investigate whether other cellular sites might contribute in association with, or in addition to, hepatic modifications.

Lastly, it can be said with certainty that the risks of inherent endotoxaemia

following surgical intervention should not be considered to be a deterrent in patients in whom splenectomy may be indicated as an adjunct to the management of a clinical problem. In addition, endotoxin may be used just as effectively in splenectomized subjects as in patients possessing a normal spleen.

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