Effect of Splenectomy on the Immune Response in the Lizard, *Calotes versicolor*

While the importance of the spleen in immune reactions has been well established for most vertebrate classes, the effect of splenectomy has been shown to vary depending on the species, on the antigen, on the route of administration of antigen, and on the time of splenectomy[1]. In contrast to information available for teleosts[2,3] and elasmobranchs[2], and in contrast also to studies on effects of splenectomy in birds[4,5], phylogenetic information on the effects of splenectomy in reptiles are not available. The present experiments were therefore carried out as part of a systematic analysis of the immune capacity of the lizard, *Calotes versicolor*[6–9].

Material and methods. Male adult lizards weighing 25–45 g were maintained at ambient temperatures ranging from 20 °C to 28 °C. Methods of feeding and maintenance of the animals in the laboratory were as described earlier[8]. Animals were anaesthetized by injection of 40 mg sodium pentobarbitone/kg body weight[9]. The trunk region was sterilized with ethanol, an incision made on the left side of the abdomen between the ribs, the stomach turned aside and the spleen removed. The incision was closed by applying collodion to the cut edges after spraying the wound area with sulphathiazole powder ('Cibazol', Ciba Ltd., India). Sham splenectomy was carried out by following all surgical procedures except actual removal of the spleen. Completeness of the operation was ascertained both by microscopic examination of the ablated spleen and by autopsy at the end of the investigation.

Animals were given an i.p. injection of 0.1 ml of 25% sheep red blood cells (SRBC) either 7 days after splenectomg (Group I), 2 h after splenectomy (Group II) or 7 days before splenectomy (Group III). Seven animals in Group II (IIa) were at the same time also injected with autologous spleen cells suspended in 0.3 ml of phosphate buffered saline. Serum analysis was performed 21 days after immunization.

Spleen cell suspensions were prepared in 10% serum-saline, and viability of these cells was determined by trypan blue dye exclusion. To detect the presence of plaque-forming cells (PFC), the technique of CUNNINGHAM and SZENBERG[10] was followed, with appropriate modifications[7]. Haemolysin (HL) and haemagglutinin (HA) levels were determined individually on all sera, using serial 2-fold dilution of inactivated serum samples. Mercaptoethanol (2-ME) sensitivity of the antibody was measured as described by GLOBERSON and AUERBACH[11].

Results. As shown in the Table, all animals immunized after splenectomy (Ie and IIe) failed to show serum antibody response, in contrast to sham-operated controls (Ic and IIc). Intraperitoneal injection of isolated autologous spleen cells (IIa) failed to restore the immune response to SRBC. Low HL and HA titres were found in 3 of the 8 lizards splenectomized 1 week after injection of antigen. Assay for PFC in the spleens removed during splenectomy of this group indicated a range of 40–343 PFC/10^6 leukocytes.

Attempts were made to restore the immunological capacity of splenectomized lizards by implanting the autologous spleen in different implantation sites, but no viable grafts were recovered. In view of these results, partial splenectomy was carried out, thus assuring both equivalent operational shock and partial presence of spleen for subsequent assay. Animals in this group were immunized 7 days following the operation and examined for PFC 14 days later. 6 out of 7 animals of this group responded well, with an average of 654 PFC/10^6 leukocytes (range of 10–1333).

All positive serum samples were tested for mercaptoethanol sensitivity. No antibody activity was detected after treatment with 2-ME.

Discussion. The results indicate that in the lizard, *Calotes versicolor*, no serum antibody response to SRBC can be detected in the absence of the spleen. This finding is in sharp contrast to the condition existing in higher vertebrates. In mammals, splenectomy did not abrogate the immune response to i.p. injected antigen, although in the case of i.v. presented antigen, especially with low doses, the response was diminished[1,12,13]. In the chicken, splenectomy followed by i.v. immunization with SRBC led to the appearance of antibody-producing cells in bone marrow[4]; this finding supports the earlier extensive studies of ROSENQUIST and WOLFE[5] who, using BSA as

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Effect of splenectomy on haemolysin (HL) and haemagglutinin (HA) response in the lizard following i.p. administration of sheep red blood cells

Group	Treatment	Time of injection of SRBC	No. positive/ total (HL)	HL titre (reciprocal)	No. positive/ total HA	HA titre (reciprocal)
I e	splenectomy	7 days after operation	0/7	—	0/7	—
I c	sham		8/11	160, 80, 40, 80, 80, 640, 640, 640	6/11	10, 10, 10, 20, 5, 40
II e	splenectomy	2 hours after operation	0/9	—	0/9	—
II a	splenectomy + spleen cells		0/7	—	0/7	—
II c	sham-operated		2/5	1280, 320	1/5	40
III e	splenectomy	7 days before operation	3/8	5, 10, 5	2/8	5, 5
III c	sham-operated		4/6	40, 1280, 640, 20	2/6	80, 40

antigen, demonstrated that splenectomy in the chicken only delayed the day of peak titre, but did not diminish the total response.

In the present study, a weak antibody response was noticed when splenectomy was performed 7 days after immunization. However, the low antibody titres were within the range produced by normal animals of this species on the 7th day following immunization, suggesting that the observed antibody may have been produced prior to splenectomy and simply maintained in the blood in the ensuing 2 weeks. The presence of PFC in the spleen at the time of splenectomy supports this interpretation.

Our observations on the total ablation of reaction to SRBC after splenectomy is reinforced by the observation of KANAKAMBIKA[6] who could detect PFC only in the blood and spleen of immunized lizards, but not in any other tissue tested. Similar observations have been made by ROTHE and AMBROSIUS in Testudo[14]. Further studies involving other reptilian species, other antigens, and alternate routes and schedules of immunization are required, however, before generalizations concerning reptilian spleen functions can be attempted[15].

Zusammenfassung. Nach Entfernung der Milz kann die Eidechse *Calotes versicolor* nicht mehr gegen Immunisierung mit Schafserythrozyten reagieren. Wenn die Milz aber nur teilweise entfernt wird, ist noch eine völlig normale Reaktion vorhanden.

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Inhibition of the Primary Immune Response Against Sheep Red Blood Cells by Short-Term Reticulo-Endothelial System Blockage Experiments in Mice

The possible suppressive effect of the immune response by 'blockage' of the reticulo-endothelial system with particles is known (CRUCHAUD[1]). It is debatable, however, whether the inhibition is caused by the uptake of the particles by the RES cells or whether it is due to direct action on the surface membrane. Electron microscopic studies have shown that thorotrast is taken up by spleen cells about 20 min after the i.v. injection and most of the thorotrast at that time still covers the surface membrane (WEISS[2]). We studied the question in short-term experiments using thorotrast as blocking agent and sheep red blood cells (SRBC) as the antigen.

Methods. 0.2 ml of thorotrast were injected i.v. into adult female and male mice. The thorotrast injection was related to the antigen injection in the following way: A) 15 sec, 30 sec, 1, 2, 5, 15, 45, 60, 90 and 120 min prior to the antigen injection, 8×10^8 SRBC. B) Together with the antigen. C) 15 sec, 30 sec, 1 min, 5 min and 2 h after the antigen application. The animals were sacrificed 72 or 96 h after the immunization and the immune response evaluated by counting the plaque forming cells in the Jerne test; 11 different experimental groups were set up, each of them involving 12–92 mice. The results were reproduced at least 3 times. The findings were statistically evaluated by variance analysis in single classification according to the model $\gamma_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ and by linear comparison of the different experimental groups with the control mice, which were immunized against SRBC without any thorotrast injection. This linear comparison was performed according to the test of Scheffe. The numeric calculations were done on IBM-7040-computer of the Calculation Center at the University of Freiburg, West Germany, Dr. Bloedhorn.

Results. The injection of thorotrast 15–30 sec, 1–5 min and 30–90 min prior to the injection of antigen inhibited the appearance of plaque-forming cells. The inhibition was statistically highly significant, $p \leq 0.001$, corresponding to a probability of error of 0.1%. The injection of thorotrast simultaneously with the antigen or 15 sec to 2 h after the antigen injection did not influence the number of plaque-forming cells compared with the control animals which obtain only SRBC and no thorotrast at all.

These results are compatible with the assumption that the inhibitory effect observed is due to an action of thorotrast on the surface of the immune competent cells in the spleen of mice. Because of this, the antigenic determinants are not able to meet their membrane receptor sites. Whether the action of thorotrast is merely a physical coating of the cell or a chemico-physical interaction with the cell surface membrane cannot be deduced from these experiments. The experimental model described can be used for studies on cell membrane in its functional relation to the antibody response[3].

Zusammenfassung. Mäuse wurden gegen Schaferythrozyten immunisiert und die Immunreaktion im Plaque-Test von Jerne gemessen. Erhalten die Tiere unmittelbar vor der Antigeninjektion Thorotrast, so kommt es zur statistisch hochsignifikanten Hemmung der Antikörper bildenden Zellen.

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