Zusammenfassung. Gebiete aus dem Hensen'schen Knoten wurden implantiert, wobei dessen DNA-RNA mit Uridin-H^3 markiert wurde. Die induzierten Neuralanlagen enthielten kaum markierte Substanzen, und wenn, dann nur im Bereich der Kontaktstellen, woraus folgt, dass andere Substanzen für die Induktion verantwortlich sein müssen.

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Thrombin-Induced 'Macrophage Disappearance Reaction' in Mice

As previously shown in guinea-pigs, macrophages disappear from the peritoneal fluid of sensitized animals after reinjecting the animal with specific antigen[1]. This macrophage disappearance reaction (MDR) is one of the in vivo manifestations of delayed hypersensitivity. Endotoxin elicited a similar effect[1] which was thought to be the result of the natural hypersensitivity state to endotoxin[2,3]. FORBES[4] demonstrated that endotoxin treatment caused the disappearance of macrophages from the peritoneal fluid of mice, too. As anticoagulants, heparin and warfarin, inhibited the MDR in guinea-pigs[5], it was anticipated that the coagulation system, or at least some of its components, plays a role in this in vivo reaction. In the present study the effect of intraperitoneal thrombin injection was examined on the peritoneal differential cell count of mice.

Materials and methods. Throughout the experiments 8–14-week-old randomly bred male BALB/c mice were used, and in each series of experiment the treated and control animals were from the same age. At various intervals after giving thrombin or saline i.p., the animals were anesthesized with ether and the peritoneal cavity was washed out with 3 ml of TC 199 solution containing 5 units/ml heparin and 2% normal inactivated rabbit serum. Total and glass-adherent cell-count was determined. The glass-adherent cells were separated as follows: small chambers were mounted on microscope-slides; the walls of the chambers were formed by two half cover slips of 0.4 mm thickness and this was covered by a Bürker cover slip. The cell suspension was incubated in these chambers for 60 min at 37 °C in a humid atmosphere, then the cover-slips were removed and the slides were allowed to stand in a reverse position for 10 min in a TC 199 solution.

After staining with Giemsa-stain, absolute and differential cell counts were determined. The adherent mononuclear cells were regarded as macrophages and the number of lymphocytes was calculated from the difference of the total and adherent cell numbers.

For fibrinogen determination[6], the peritoneal cavity was washed out with 3 ml of citrate solution (0.38% Na-citrate, 0.8% NaCl).

Results and discussion. Table I shows that 2 (NIH) units of thrombin (Topostasin, Hoffmam-La Roche) did not appreciably decrease the peritoneal fibrinogen content, although the total cell count diminished, which could be prevented by heat-inactivating the thrombin. 10 U of thrombin significantly reduced both the fibrinogen level and the cell number.

Table II shows the effects of 2 and 10 U of thrombin on the differential cell count of the peritoneal cells. The great majority of the macrophages disappear from the peritoneal fluid, while the number of lymphocytes diminishes less markedly. The effect of 10 U of thrombin lasts at least for 5 h and after 28 h the cell picture becomes normal again (Table III).

Table I. Effect of thrombin on the peritoneal fibrinogen level and cell count

Treatment[a]	No. of animals	Fibrinogen (μg)	Cell-count ($\times 10^6$)
Salt	6	122 ± 8	14.2 ± 1.5
Thrombin, 2 U (inactivated[b])	6	127 ± 18	14.0 ± 1.2
Thrombin, 2 U	6	122 ± 36	10.5 ± 2.0
Salt	5	86.5 ± 6	13.9 ± 1.8
Thrombin, 10 U (inactivated[b])	5	114 ± 42	13.1 ± 2.4
Thrombin, 10 U	5	21 ± 7	8.3 ± 2.0

[a] 1 h before sacrificing the animals. [b] Thrombin was inactivated at 70 °C for 15 min.

Table II. Effect of thrombin on the peritoneal cell-count

Treatment[a]	Cell-count $\times 10^6$ per mouse				
	Total	Adherent	Macrophages	Lymphocytes	Granulocytes
Salt	16.9 ± 0.2	5.23 ± 0.65	4.90 ± 0.52	11.6 ± 2.5	0.33 ± 0.11
Thrombin, 2 U	8.4 ± 2.1	1.26 ± 0.26	1.01 ± 0.27	7.1 ± 1.9	0.25 ± 0.05
Thrombin, 10 U	8.1 ± 0.7	0.81 ± 0.16	0.57 ± 0.24	7.3 ± 0.5	0.24 ± 0.08

[a] 1 h before sacrificing the animals; 5 mice per group.

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Table III. Effect of thrombin on the peritoneal cell-count

Treatment[a]	Interval[b] (h)	Cell-count × 10^6 per mouse Total	Adherent	Macrophages	Lymphocytes	Granulocytes
Salt	1	20.4 ± 1.4	4.70 ± 1.20	4.50 ± 0.33	15.7 ± 1.2	0.20 ± 0.03
Thrombin	1	10.0 ± 1.3	0.66 ± 0.09	0.46 ± 0.12	9.3 ± 0.5	0.20 ± 0.02
Salt	5	20.2 ± 2.4	3.99 ± 0.62	3.85 ± 0.63	16.2 ± 2.2	0.14 ± 0.02
Thrombin	5	13.8 ± 1.0	0.78 ± 0.16	0.57 ± 0.14	13.0 ± 1.0	0.21 ± 0.05
Salt	28	19.4 ± 1.4	4.82 ± 0.46	4.54 ± 0.55	14.6 ± 1.1	0.28 ± 0.04
Thrombin	28	17.4 ± 1.2	4.23 ± 0.81	3.67 ± 0.41	13.1 ± 1.0	0.56 ± 0.33

[a] 5 mice per group; The dose of thrombin was 10 NIH units per mouse. [b] Interval between treatment and sacrificing the animal.

The reason for the disappearance of macrophages from the peritoneal fluid may be interpreted as follows: the fibrinogen-fibrin conversion goes on first of all on the surface of macrophages (and large lymphocytes) and the fibrin or some intermediary product fixes these cells onto the peritoneal wall. Other mechanisms are also possible. Several data indicate the role of the clotting system both in non-specific inflammatory[7,8] and in delayed hypersensitivity reactions[5,9,10].

The MDR is thought to have some relation to the production of migratory inhibitory factor (MIF)[11,12].

It seems from the literature that there is a significant difference in sensitivity between the in vivo (MDR) and in vitro assays (inhibition of migration) of delayed hypersensitivity reactions. NELSON[1] found about 0.1 µg of PPD to be necessary for eliciting MDR in guinea-pigs and observed a significant effect even 1 h after injecting antigen. In the in vitro assays of MIF, however, generally 10–100 µg/ml PPD were used (1 µg/ml was ineffective[13]) and the minimal incubation time necessary to give positive results is about 6 h[13].

This discrepancy in sensitivity between the in vivo and in vitro tests might well be explained by the in vivo activation of the coagulation mechanism as a non-specific amplifier system. The granulocytes, which are often present in inflammatory area, may also provide procoagulation (tissue) factors[14–16].

Zusammenfassung. Intraperitoneale Gabe von Thrombin verursachte ein praktisch völliges Verschwinden der Makrophagen aus der Peritonealflüssigkeit, beeinflusste aber kaum die Zahl der Lymphozyten.

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Influence of Classical Immunosuppressants on Spleen Colony Formation in Mice

In diminishing immunological response to allograft, cyclophosphamide[1,2], immuran[3], and cortisone[4] are widely used. To compare depressive action of these drugs on hemopoietic regeneration, a study of their influence on formation of endogenous spleen colonies, after sublethal whole body irradiation, was undertaken. Such studies may be of significant importance, as immunosuppressant is also recommended in bone marrow transplantation in order to diminish the incidence of secondary disease[5]. To make the comparison more accurate, cyclophosphamide, immuran, and cortisone doses, giving approximately the same spleen involution after treatment of 600 R irradiated mice, were chosen in pilot experiments.

Materials and methods. Female Swiss mice (6–8 weeks old) were exposed on day 0 to 600 R whole body irradiation and were divided into 4 groups, each group consisting of 10 animals. In 3 groups they were given on −1, 1, 3, 5, and 7th day respectively: 1 mg cortisone acetate (Roussel, London) s.c., 1.5 mg immuran (Burroughs, London), and 2 mg cyclophosphamide (VEB Ankelwerk, Rudolstadt) intraperitoneally. The 4th control group received 0.1 ml saline, according to the same schedule. On the 10th day after irradiation, all mice were weighed and killed. Spleens were weighed and discrete nodules on the surface of the spleens, described as Colony Forming Units[6], were counted. From every mice one femur was removed, all bone marrow cells were washed through and nucleated cells were counted.

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