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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Influence of the treatment with immunosupressants on the endogenous spleen colony formation after 600 R whole body irradiation in mice

	Control Mean	SD	CY[a] Mean	SD	Immuran Mean	SD	Cortisone Mean	SD
Body weight	20.3	2.7	15.6	1.2	16.0	1.8	18.1	2.0
Spleen weight	76.3	29.7	27.5	10.3	32.3	12.1	32.6	7.0
Number of spleen nodules	27.7	7.8	0–3[b]	2.1	1.9	1.5	10.0	4.7
Number of nucleated marrow cells per femur $\times 10^6$	6.0	3.5	0.2	0.1	0.7	0.4	2.1	1.2

[a]Cyclophosphamide.[b] Only extreme values are presented. One spleen in this group contained 6 nodules, which was not comparable with spleen nodule numbers of other mice in this group (see discussion).

Results and discussion. The results of this experiment are given in the Table. Immuran and cyclophosphamide treatment of sublethally irradiated mice resulted in significantly more severe depression of endogenous colony formation than did cortisone treatment. Conforming to the number of spleen colonies, bone marrow cellularity was more depressed in immuran and cyclophosphamide groups as compared with cortisone group. According to PETROV et al.[7], who studied mitostatic and cytostatic effects of various immunosuppressants, cyclophosphamide had high range of selective lymphotoxic action, while immuran was devoid of such selectivity. These 2 drugs, used in our experiment in doses giving the same spleen involution, had almost the same effect on spleen colony formation, and bone marrow cellularity was even significantly higher in immuran-treated than in cyclophosphamide-treated mice.

The need for these drugs, displaying strong immunosuppressive activity without severe depression of the hemopoiesis, encouraged us to undertake this study. In choosing the doses of the drugs, we were aware of the possibility that the same spleen involution produced by different immunosuppressants does not necessarily cause the same depression of allograft response. Different mechanisms of action seem to be involved. Cortisone is, for example, expected to suppress mainly thymus-dependant[8], and cyclophosphamide non-thymus-dependant[9] areas of the spleen. The doses, giving the same depression of allograft response, should be applied in further comparative studies.

Zusammenfassung. Nachweis eines Hemmeffektes immunosupressiver Mittel (Cortison, Cyclophosphamide und Immuran) bei Mäusen nach UV-Bestrahlung auf die hämopoetische Regeneration (Milzkolonien und Knochenmarkzellen).

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A Quantitative Difference in the Immune Response to Sheep Red Cells between Rat Strains

Important quantitative differences in humoral antibody production have been observed between mouse strains following similar antigenic stimulation[1], but nothing of the sort has as yet been reported in the rat. The present study was undertaken to explore the capacity for haemolysin production in several rat strains upon challenge with sheep red blood cells (SRBC).

Materials and methods. Rats. Adult female rats used in this study (Agus, PVG/c, Okamoto, August, Long-Evans, Sherman, BN, Gunn, Fischer, LOU, OFA, CD, Wistar R) were obtained from the sources listed in Table I. All animals had been housed and fed in a similar fashion. All rats but those of the OFA and CD strains were inbred lines. LOU rats and the following Fl hybrids: (August × LOU), (Okamoto × LOU) and (CD × LOU) were raised at this laboratory. Histocompatibility within the LOU strain had been assessed by skin grafting.

Antigen. Sheep erythrocytes (Institut Pasteur, Paris), were washed 3 times in phosphate buffered saline (pH 7.2) and made up to a 2% suspension prior to injection. The animals were immunized by a single i.p. injection of 0.5 ml of this suspension, corresponding to 2×10^8 erythrocytes. six days after immunization, blood was drawn from the retro-orbital sinus.

Haemolysin titration. Blood was allowed to clot at room temperature and the serum was centrifuged after retraction of the clot. Sera were inactivated by heating to 56 °C for 60 min. Sera from non-immunized rats were used as controls. A microtitration apparatus was used (Microtiter-Cooke Engineering Company). Serial 2-fold dilutions of serum with 0.9% NaCl were prepared in titration plates, the initial dilution being 1:2.

To 0.05 ml of diluted serum was added 0.025 ml of a 2.5% suspension of washed SRBC. The plates were incubated at 37 °C for 30 min, after which 0.025 ml of 1:10 diluted guinea-pig serum was added, followed by another period of incubation at 37 °C for 1 h.

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