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.

[1] W. F. BARTH, C. L. MCLAUGHLIN and J. L. FAHEY, J. Immun. *95*, 781 (1965).

Table I. Rat strains and source

Agus PVG/c	MRC Laboratory Animals Centre Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, Great-Britain.
August Long-Evans Sherman	Centre de Sélection et d'Elevage des Animaux Laboratoire, C.N.R.S., F-54 Orléans-la-Source, France.
B.N. Gunn	Proefdierencentrum K.U.L., De Croylaan 34, B-3030 Heverlee, Belgique.
C.D.	Charles River France S.A. B.P. 29, F-76 St-Aubin-les-Elbœuf, France.
LOU	Animalerie de l'Ecole de Santé Publique, U.C.L., Avenue Chapelle-aux-Champs 4, B-1200 Bruxelles, Belgique.
OFA Fischer	Centre de Recherche et d'Elevage des Oncins, IFFA-Credo, F-69 St-Germain-sur-l'Arbresle, France.
OKAMOTO	Continental Pharma, Stw. Haacht 30, B-1830 Machelen, Belgique.
Wistar R	Département de Radiobiologie, Centre d'Etude et de l'Energie Nucléaire, CEN-SCK, B-2400 Mol, Belgique.

Table II. Geometrical mean (×2) dilution titres of sera giving complete and incomplete haemolysis

Strains	No Rats	Complete haemolysis	Incomplete haemolysis
Agus	5	< 1 [a]	3.2 (3–4) [b]
August	5	1.2 (0–2)	5.6 (5–6)
BN	5	< 1	5.8 (0–8)
CD	5	< 1	2.8 (2–3)
Fischer	5	< 1	1.6 (0–3)
Gunn	5	< 1	6.4 (1–10)
Long-Evans	5	< 1	5.2 (0–9)
LOU	5	< 1	9 (7–10)
OFA	5	3.6 (2–5)	8 (6–10)
PVG/c	5	< 1	4.6 (1–8)
Sherman	5	< 1	2.8 (1–4)
Wistar R	5	2.2 (1–2)	6 (4–7)
F1 (August × LOU)	5	< 1	10.6 (9–11)
F1 (CD × LOU)	5	< 1	8.8 (5–11)
F1 Okamoto × LOU)	5	< 1	6.6 (2–10)

[a] Haemolysis still incomplete with 1:2 diluted rat serum. [b] Range.

The highest dilution of serum producing complete haemolysis was read as the end point, incomplete haemolysis also being taken into account. The titres were recorded as the reciprocals of these serum dilutions.

Results. The highest mean serum dilution giving complete or incomplete haemolyse are listed in Table II. The results represent geometrical mean (×2) dilutions titres; the range is also indicated. With the test conditions employed, complete haemolysis was observed only with 3 of the rat strains, viz. OFA, Wistar R and August. The highest antidody response was observed with OFA rats. All strains responded with humoral antibodies to SRBC but important strain differences were evident.

Discussion. The discrepancies observed between the mean antidoby responses of different rat strains to the same immunogenic challenge with sheep erythrocytes strongly suggest that a genetic factor is involved in this type of immune response. This hypothesis is also consistent with the observation that hybrids between a good responder strain (August) and a moderate responder strain (LOU), or between a moderate (LOU) and a poor responder strain (CD) performed in a manner rather similar to that of the better of the two parent strains.

In this respect our finding with sheep erythrocytes given to rats is reminiscent of those that have been observed with other antigens in various species, viz. the mouse, rat and guinea-pig[2–6]. In the present case the response was not an all-or-not matter, but this may have been due to the fact that the sheep erythrocyte antigen was not administered in a limiting dose.

Résumé. Des rats appartenant à plusieurs souches ont été immunisés par injection i.p. de 2×10^8 globules rouges de mouton. La mesure du titre des haemolysines réalisée 6 jours plus tard montre des différences quantitatives selon les souches. Une réponse importante n'a pu être décelée que pour 3 d'entre elles (August, OFA et Wistar R).

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Diffusible Factor of Thymus is Responsible for the Recovery From Some Effects of Heterologous Antilymphocyte Serum

The target of heterologous antilymphocyte serum (ALS) are thymus dependent lymphocytes[1] but the role of the thymus in the mechanism of action of ALS remains uncertain. Thymectomy potentiates the immunosuppressive effect of ALS[2], a fact not confirmed by others[3]. Jeejeebhoy and Singla[4] postulated that the delay in the recovery from ALS effects is due to the presence of free immunosuppressive antibodies in the blood of