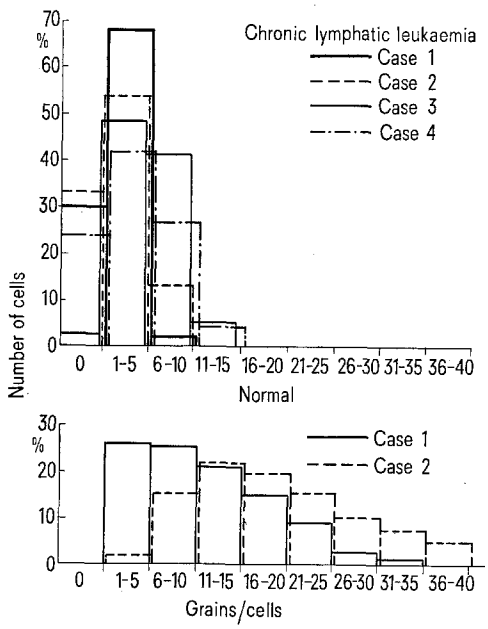
Lymphocyte Actinomycin Binding Capacity in Chronic Lymphocytic Leukaemia

Methods for the identification of cell surface immunoglobulin have been used to distinguish marrow-derived (B) and thymus-derived (T) lymphocytes in the normal circulating blood; they are involved in humoral response and cell immunity, respectively. WILSON and NOSSAL[1] and PERNIS[2] have produced independent evidence for the ability of lymphocytes, in chronic lymphatic leukaemia (CLL), to bind anti-immunoglobulins, but to a much lesser extent than the normal B lymphocyte. This has been taken as evidence that the CLL lymphocyte is of the B type, although atypical with respect to its lower immunoglobulin content.



Grain count frequency histogram for normal blood (2 cases) and chronic lymphatic leukaemia lymphocytes (4 cases) following ^{3}H-actinomycin labelling.

This below-normal immunoglobulin content may be considered as the result of disturbed genetic activity, i.e. of genetic repression. It was therefore decided to compare actinomycin binding, which can be taken as an expression of genetic activity (as shown by the increased binding observed in normal lymphocytes by DARZYNKIEWICZ et al.[3] after phytohemagglutinin stimulation) in CLL and normal lymphocytes.

Actinomycin binding was therefore evaluated in 4 cases of CLL and in 2 normal subjects, by means of an in situ method, based on the measurement, after autoradiography, of ^{3}H-actinomycin binding to the nuclei of smeared and fixed cells[4].

The Figure shows that CLL lymphocytes were either incapable of binding actinomycin D, or displayed binding values considerably below those found for the normal lymphocyte population by GAVOSTO and MASERA[5].

These data, with respect to actinomycin binding as an expression of genetic activity, offer further evidence of the abnormal character of CLL lymphocytes. Depressed genetic activity might be directly responsible for their decreased immunoglobulin content[1].

Zusammenfassung. Lymphocyten von 4 Fällen chronischer lymphatischer Leukämie zeigten ein vermindertes Bindungsvermögen für ^{3}H-Actinomycin.

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[1] J. D. WILSON and G. J. V. NOSSAL, Lancet *1971*, 788.
[2] B. PERNIS, L. FORNI and L. AMANTE, Ann. N.Y. Acad. Sci. *190*, 420 (1971).
[3] Z. DARZYNKIEWICZ, L. BOLUND and N. R. RINGERTZ, Expl. Cell Res. *55*, 120 (1969).
[4] J. BRACHET and N. HULIN, Nature *222*, 481 (1969).
[5] F. GAVOSTO and P. MASERA, to be published.
[6] This work was supported by C.N.R., Rome.

Hematopoietic Changes in NMRI Mice After the Intravenous and Subcutaneous Injection of *Bordetella pertussis* Vaccine

The development of pronounced leukocytosis is one of the prominent features induced by killed pertussis organisms (PO) in man and experimental animals[1,2]. It is characterized by the increase in both the blood granulocyte[3-5] and lymphocyte[6-8] counts. According to MORSE[7] the maximum is reached 4 days after the i.v. injection of PO and returns to normal by the 14th day. The same author reported that the route of injection is of crucial importance for the production of PO-mediated leukocytosis. The i.v. route was the most effective, whilst the s.c. route was ineffective. Although no plausible explanation could be given for this phenomenon[7], there evidently exists a striking similarity to PO-mediated histamine sensitization which likewise was not produced by s.c. application[1,2,9-11]. In our own experience, however, the s.c. injection of PO effects both increase in the blood leukocyte counts and marked splenomegaly.

Young adult female NMRI mice weighing 20–24 g were used. This strain of specifically pathogen-free mice was obtained from the Central Institute for Laboratory Animals in Hannover, Germany. In experiment A) 4 groups of mice were used. The animals of group I functioning as controls were treated by a single i.p. injection of 0.1 ml of phosphate-buffered saline, pH 7.2 (PBS). The other groups received 0.1 ml of a *Bordetella pertussis* vaccine (phase 1, not adsorbed but treated with merthiolate at a dilution of 1:10,000 for 30 min at 56°C; OP-no. 678a of the Behring-Werke, Marburg, Germany) containing 3×10^{10} PO per ml. It was administered either by the i.p. (group II), i.v. (group III) or s.c. (group IV) route. At different intervals after treatment 5 to 6 mice out of each group were sacrificed, and the spleens were removed. Subsequently, the wet spleen weights were determined gravimetrically. The spleen index is defined as wet spleen weight/body weight (mg/g). The data presented in Table I show that splenomegaly was most pronounced following the i.p. and i.v. injection of PO. The s.c. injection of PO also led to significant splenome-

Table I. Spleen indexes[a] determined after i.p. (group II), i.v. (group III) or s.c. (group IV) injection of 3×10^9 pertussis organisms, as compared to controls (group I)

Days after primary immunization	Spleen indexes determined						
	Group I	Group II		Group III		Group IV	
	$\bar{x} \pm$ SE[b]	$\bar{x} \pm$ SE[b]	P	Mean value $\pm$ SE[b]	P	Mean value $\pm$ SE[b]	P
5	5.0 ± 0.28	14.2 ± 0.81	< 0.001	14.7 ± 0.81	< 0.001	10.1 ± 0.68	< 0.001
6	5.6 ± 0.22	18.0 ± 0.47	< 0.001	15.2 ± 0.84	< 0.001	13.0 ± 1.64	< 0.001
11	6.2 ± 0.41	17.5 ± 1.85	< 0.001	12.9 ± 0.74	< 0.001	12.6 ± 0.94	< 0.001
15	6.2 ± 0.37	10.4 ± 0.76	< 0.005	8.9 ± 0.84	< 0.05	9.0 ± 1.21	< 0.05
28	6.1 ± 0.07	8.5 ± 0.35	< 0.005	8.0 ± 0.38	< 0.005	8.4 ± 0.60	< 0.05

[a] Spleen indexes are defined as: wet spleen weight/body weight (mg/g). [b] mean value and standard error of 5–6 spleen indexes. P, statistical significance.

galy. Since a close positive relationship has been demonstrated between splenomegaly and PO-mediated blood leukocytosis[12], the s.c. injection of PO might effect also significant blood leukocytosis.

The experiment B) was carried out to test this effect. 3 groups of mice were used. The animals of group I received 0.1 ml of PBS by the i.v. route, whereas mice of the other 2 groups were treated with 0.1 ml of the *B. pertussis* vaccine containing 3×10^9 cells. The vaccine was injected either by the i.v. (group II) or s.c. (group III) route. Shortly before treatment and at different intervals thereafter, blood was sampled from the lateral tail vein. The quantitative determinations of both the blood leukocyte and erythrocyte counts were done with an electronic cell counter (Coulter Counter, model F). Bloods-mears were prepared and stained according to the procedure of Pappenheim. 100 cells per blood-smear were identified.

According to Table II the peripheral blood of normal NMRI mice contains 10,000 to 15,000 leukocytes per mm^3

[1] J. Munoz, in *Microbial Toxins* (Eds. S. Kadis, Th. C. Montie and S. J. Ajl; Academic Press, New York and London 1971), vol. IIa, p. 271.
[2] J. Munoz and R. K. Bergman, Bact. Rev. *32*, 103 (1968).
[3] W. L. Bradford, H. W. Scherp and M. R. Tinker, Pediatrics *18*, 64 (1956).
[4] R. A. Hammerstrom and R. D. Stoner, J. Lab. clin. Med. *62*, 985 (1963).
[5] I. A. Parfentjev and E. E. Manuelidis, Fedn. Proc. *15*, 607 (1956).
[6] K. E. Fichtelius and O. Hassler, Acta path. microbiol. scand. *42*, 189 (1958).
[7] S. I. Morse, J. exp. Med. *121*, 49 (1965).
[8] E. Ambs, Annls paediat. *206*, 102 (1966).
[9] H. B. Maitland, R. Kohn and A. D. Macdonald, J. Hyg. Camb. *53*, 196 (1955).
[10] S. Malkiel, B. J. Hargis and S. M. Feinberg, J. Immun. *71*, 311 (1953).
[11] J. Munoz, Fedn. Proc. *23*, 404 (1964).
[12] H. Finger, Ph. Stein, G. Beneke, P. Emmerling and L. Plager, Z. mikrobiol. ImmunForsch. *157*, 265 (1972).

Table II. Erythrocyte and leukocyte counts, as determined in the peripheral blood of mice shortly before and at different times after the i.v. (group II) or s.c. (group III) injection of 3×10^9 pertussis organisms[a]

Time after treatment (h)	Mouse-group	Leukocytes			Erythrocytes per mm^3 blood[b]
		Total per mm^3 blood[b]	% Lymphocytes	% Granulocytes	
0[c]	II	14,148 $\pm$ 1,681	87	13	6,702,000 $\pm$ 307,000
	III	10,875 $\pm$ 867	76	24	6,408,000 $\pm$ 293,000
24	II	29,668 $\pm$ 4,817	71	29	5,380,000 $\pm$ 303,000
	III	14,034 $\pm$ 1,011	76	24	6,329,000 $\pm$ 77,000
48	II	34,509 $\pm$ 3,438	65	35	4,871,000 $\pm$ 238,000
	III	16,392 $\pm$ 1,795	78	22	5,380,000 $\pm$ 121,000
72	II	50,425 $\pm$ 5,714	80	20	4,343,000 $\pm$ 281,000
	III	19,538 $\pm$ 969	74	26	4,520,000 $\pm$ 318,000
96	II	63,361 $\pm$ 5,690	68	32	4,243,000 $\pm$ 125,000
	III	28,697 $\pm$ 1,722	54	46	4,807,000 $\pm$ 259,000
120	II	61,127 $\pm$ 7,196	65	35	3,980,000 $\pm$ 384,000
	III	44,965 $\pm$ 2,769	45	55	3,993,000 $\pm$ 249,000
240	II	18,264 $\pm$ 1,748	60	40	6,859,000 $\pm$ 173,000
	III	25,108 $\pm$ 1,945	50	50	6,442,000 $\pm$ 193,000
360	II	12,794 $\pm$ 882	74	26	6,885,000 $\pm$ 163,000
	III	14,189 $\pm$ 961	71	29	6,961,000 $\pm$ 264,000

[a] No noteworthy changes in the blood leukocyte and erythrocyte counts were found during the time of observation in mouse group 1 treated with PBS. [b] Mean values and standard errors from 8 mice per point. [c] Values determined in blood samples taken shortly before treatment with pertussis organisms.

with approximatively 80% lymphocytes. This is in accordance with the data of MORSE[7] and with our own findings[13]. After i.v. injection of PO the blood leukocyte counts increased rapidly, and reached a peak on the days 4 and 5. This was found to be due to a multiplication of both lymphocytes and granulocytes (Table II). 15 days after treatment, normal leukocyte counts were determined again. Pronounced leukocytosis became also detectable after s.c. injection of PO. But in comparison with the i.v. route, the development of leukocytosis appeared to be considerably delayed (Table II). This may explain why MORSE[7] did not demonstrate blood leukocytosis after the s.c. injection of PO, since he measured the response 1, 4 and 7 days after treatment.

On the basis of hematocrits and reticulocyte counts determined in the peripheral blood of NCS mice, the injection of PO was not found to be followed by a significant change in cells of the erythrocyte series[7]. This is not in full accordance with the findings of FRUHMAN[14] who demonstrated a doubling in reticulocyte percentages 7 days after the injection of PO into CF No. 1 mice, although the concentrations of circulating erythrocytes at this time were in the normal range. On the basis of further cytologic and ferrokinetic studies, the conclusion was drawn that the injection of *B. pertussis* leads to marked increase in splenic erythropoiesis accompanied by a decrease in bone marrow erythropoiesis[14]. Our data show that both the i.v. and the s.c. injection of PO results in marked decrease in the erythrocyte concentrations, whereby the lowest values were obtained at the 5th day returning to normal within the following 5 days (Table II). The decrease in the erythrocyte counts is apparently due to the damage of erythrocytes, and is attributed to the toxicity of *B. pertussis*[1,2,7,13].

Zusammenfassung. Sowohl die i.v. als auch die s.c. Injektion abgetöteter Zellen von *Bordetella pertussis* führt bei Mäusen zu Splenomegalie und ausgeprägter Blut-Leukozytose, an der Lymphozyten und Granulozyten beteiligt sind. Zudem bewirkte die Injektion von *B. pertussis* eine deutliche Verminderung der Erythrocyten mit Minimalwerten am 5. Tag. Am 10. Tag und danach wurden wieder Normalwerte gefunden.

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[13] G. BENEKE, H. FINGER and P. EMMERLING, Z. Mikrobiol. Immun-Forsch. *154*, 178 (1968).
[14] G. J. FRUHMAN, Proc. Soc. exp. Biol. Med., *124*, 728 (1967).
[15] This study was supported by the Kurt- und Inge-Schuster-Stiftung.

The Fate of Polymorphonuclear Neutrophils and Mononuclear Cells During Allograft Rejection in the Urodele *Pleurodeles waltlii* Michah.

Allograft rejection in Urodeles is actually a wellknown phenomenon and has been studied in various genus and species. Chronic rejection of skin grafts described by COHEN[1-3] in *Triturus viridescens* and in other species[4], is a general phenomenon. Acute rejection remains possible in some cases. Second set grafts are rejected in an accelerated fashion. In the Urodele, *Pleurodeles waltlii*[5], survival times of skin allografts are variables: rejection occurs in 50% of the grafts 15 to 20 days after grafting in a 'sub-acute' manner and is chronic for the other grafts (20–130 days after grafting). In genetically related newts, some cases of definitive tolerance sometimes occur.

In Urodeles, the hematopoietic system is primitive. Bone marrow and lymph nodes are absent. Erythropoiesis and thrombocytopoiesis occur exclusively in the spleen, lymphocytopoiesis in the thymus and in the spleen and granulocytopoiesis in the capsular layer of the liver[6,7].

In *Pleurodeles*, thymectomy performed at the larval stages 51–52[8], 1 month before metamorphosis, produces definitive tolerance to allografts in adults[9]. Irregulars and

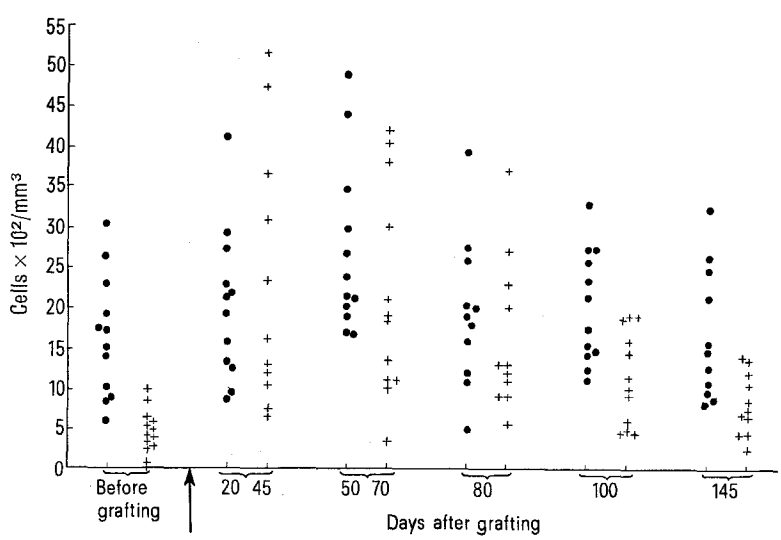

Fig. 1. The changes in the absolute numbers per mm³ of blood mononuclear cells (•) and polymorphonuclear neutrophils (+) during allograft rejection in *Pleurodeles waltlii* (12 animals).