Non-Appearance of Splenomegaly after Injection of Killed *Bordetella pertussis* Cells into Aged Mice

Many physiological changes have been observed in mice after the injection of killed *Bordetella pertussis* cells[1]. Their most prominent feature is the capacity to induce lymphocytosis[2] and to produce adjuvant activity. In mice this is regularly associated with increase in the wet and dry spleen weights[2-4], caused by an increase in the cell number and enhanced protein contents of the individual cells[4,5]. The following experiments give evidence that splenomegaly does not occur when the pertussis vaccine is injected into aged mice.

Three-month-old female NMRI mice were immunized i.p. with either 4×10^8 sheep red blood cells (SRBC) (group I) or 4×10^8 SRBC and 3×10^9 pertussis organisms (PO) (phase I, not adsorbed but treated with merthiolate at a dilution of 1:10,000 for 30 min at 56° C) (group II). For the experimental groups III and IV immunized in the same manner (group III: 4×10^8 SRBC; group IV: 4×10^8 SRBC and 3×10^9 PO) 20-month-old female NMRI mice were used. At different intervals after the immunization, 6 mice out of each group and 2 animals of the untreated controls were sacrificed and their spleens removed. Immediately after taking the spleens, their wet weights were determined gravimetrically. Usually the total number of spleen cells is determined by teasing out the cell contents of each spleen into a culture medium. Since small tissue fragments were used for histological studies and for the quantitative determination of 19S and 7S hemolysin producing cells, the total cell counts of the spleens were calculated from the deoxyribonucleic acid (DNA) contents. Assuming that there exists essentially no polyploidy of the individual cell, and making the additional assumption that the number of cells being in the phase of DNA synthesis at the time of investigation is relatively small, the number of cells per spleen was estimated. Since most spleen cells possess a diploid DNA content of 6×10^{-12}g of DNA, those cells possessing a different DNA content can be numerically neglected[4,5]. Immediately after weighing the spleens, fragments were taken, weighed and used for the determination of DNA by the method of BURTON[6] as described elsewhere[5]. The DNA contents of the total spleens were calculated on the basis of the spleen weights. As compared to the injection of 3-month-old mice with 4×10^8 SRBC (group I), the additional injection of 3×10^9 PO leads to a significant elevation of the wet spleen weights. This effect of the *B. pertussis* vaccine is not due to a general growth process as can be seen from the wet spleen indexes (Table I). The elevation of the spleen weights is caused to a considerable extent

Table I. Spleen indexes[a] determined in the spleens of 3-month-old mice after primary immunization with 4×10^8 sheep red blood cells (SRBC) (group I) and 4×10^8 SRBC + 3×10^9 pertussis organisms (PO) (group II) as compared to those determined in 20-month-old mice after primary immunization with 4×10^8 SRBC (group III) and 4×10^8 SRBC + 3×10^9 PO (group IV)

Days after immunization	Spleen indexes determined[b]			
	Group I	Group II	Group III	Group IV
3	7.2 ± 0.43	9.8 ± 0.68	8.4 ± 2.57	7.7 ± 1.53
4	6.1 ± 0.40	11.1 ± 0.46	18.2 ± 6.51	9.6 ± 1.42
5	5.3 ± 0.39	12.8 ± 0.70	5.9 ± 0.46	13.2 ± 5.18
6	5.4 ± 0.68	13.9 ± 2.08	10.6 ± 3.16	7.7 ± 0.23
7	5.2 ± 0.27	17.0 ± 0.73	9.0 ± 2.64	8.6 ± 1.37
10	5.5 ± 0.66	15.3 ± 0.96	7.4 ± 1.41	10.4 ± 1.74
14	5.7 ± 0.42	11.8 ± 1.48	6.0 ± 1.22	6.1 ± 0.38

[a] Wet spleen weight/body weight (mg/g). [b] Mean values and standard errors of 6 spleens/group/day.

Table II. Cell counts determined in the spleens of 3-month-old mice after primary immunization with 4×10^8 sheep red blood cells (SRBC) (group I) and 4×10^8 SRBC + 3×10^9 pertussis organisms (PO) (group II) as compared to those determined in 20-month-old mice after primary immunization with 4×10^8 SRBC (group III) and 4×10^8 SRBC + 3×10^9 PO (group IV)

Days after immunization		Cell counts determined ($\times 10^3$)[a]			
		Group I	Group II	Group III	Group IV
3	(a)	566 ± 41	481 ± 53	676 ± 161	650 ± 86
	(b)	343 ± 5	248 ± 15	237 ± 17	234 ± 8
4	(a)	399 ± 21	506 ± 37	987 ± 247	679 ± 135
	(b)	305 ± 9	239 ± 7	181 ± 20	177 ± 15
5	(a)	249 ± 23	482 ± 23	502 ± 62	717 ± 179
	(b)	222 ± 21	186 ± 6	220 ± 13	176 ± 16
6	(a)	332 ± 52	958 ± 71	722 ± 153	687 ± 108
	(b)	289 ± 6	300 ± 63	213 ± 21	237 ± 42
7	(a)	294 ± 27	701 ± 46	677 ± 110	645 ± 50
	(b)	252 ± 16	210 ± 11	239 ± 23	224 ± 18
10	(a)	283 ± 38	660 ± 35	523 ± 92	737 ± 102
	(b)	230 ± 10	203 ± 5	190 ± 7	206 ± 10
14	(a)	355 ± 39	552 ± 45	419 ± 40	446 ± 41
	(b)	259 ± 10	203 ± 12	201 ± 19	209 ± 21

[a] Mean values and standard errors of 6 spleens/group/day. Cell counts are given per total spleen (a) and per 100 mg wet spleen weight (b).

by the increase in the cell counts (Table II). As determined by the Student t-test, the spleens of the mouse group II contained significantly ($p < 0.05$) more cells between the 4th and 14th day after immunization than those of the corresponding control group I. Rather reduced cell numbers were detected per 100 mg wet spleen weight in the pertussis-treated mouse group II. This suggests that the multiplication of spleen cells is not alone responsible for the elevation of the spleen weights. As to the spleen weights of 20-month-old mice, 2 findings should be especially noted: 1. The average spleen weights of the senile mice were sometimes higher, as compared to those of the 3-month-old controls. The same applies to the spleen indexes (Table I) and cell counts (Table II). 2. In the aged mouse groups III and IV the spleen weights, spleen indexes and cell counts differed considerably, as is evident from the relatively high standard errors. Contrary to expectations, the injection of PO into senile mice (group IV) did neither effect significantly the elevation of spleen weights nor spleen indexes (Table I) nor the cell numbers (Table II).

The events leading to splenomegaly after the injection of *B. pertussis* cells are unknown. According to MORSE[2], it is not due to a production of new cells. This was concluded from the finding that the mitotic figures were no more prominent in the spleens of the pertussis-treated group than in those of the corresponding controls. On the other hand, it was demonstrated by histological investigations that proliferative changes occurred in the spleens of the treated mice, these being especially pronounced in the periphery of the follicles and in the red pulp[7]. This suggests that non-appearance of the characteristic splenomegaly in senile mice treated with PO is due to: 1. reduced 'accessible reserve of small lymphocytes'[2] or its diminished capacity to be mobilized and/or 2. reduced proliferative potential of the lymphoreticular tissue.

Zusammenfassung. Die Injektion abgetöteter Zellen von *Bordetella pertussis* bewirkt bei 3 Monate alten NMRI-Mäusen eine erhebliche Zunahme des Milzgewichtes verbunden mit einem Anstieg des Gesamtzellgehaltes der Milz. Diese charakteristischen Effekte sind bei Mäusen im Alter von 20 Monaten nicht zu beobachten. Ihr Ausbleiben im Alter ist vermutlich zurückzuführen: 1. Auf eine geringere Reserve an kleinen Lymphozyten bzw. deren verminderte Mobilisierbarkeit und/oder 2. auf eine Verminderung der Proliferationsfähigkeit des lymphoretikulären Gewebes.

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[1] J. MUNOZ and R. K. BERGMAN, Bact. Rev. *32*, 103 (1968).
[2] S. I. MORSE, J. exp. Med. *121*, 49 (1965).
[3] I. A. PARFENTJEV and E. E. MANUELIDIS, Fedn. Proc. *15*, 607 (1956).
[4] H. FINGER, Int. Arch. Allergy *36*, 293 (1969).
[5] H. FINGER, G. BENEKE und P. EMMERLING, Z. med. Mikrobiol. Immunol. *154*, 23 (1968).
[6] K. BURTON, Biochem. J. *61*, 473 (1955)
[7] G. BENEKE, H. FINGER und P. EMMERLING Z. med. Mikrobiol. Immunol. *154*, 178 (1968).

Ropalocytosis - a New Abnormality of Erythrocytes and their Precursors

During the course of our studies on a case of 'Hairy Cell' leukaemia, we have observed a startling and hitherto undescribed abnormality in the form of the erythrocytes and their precursors. 'Hairy Cell' leukaemia is a rare disease characterized by the presence of numerous cell processes arising from the surface of the leukaemic cells[1-4]. The nature of the leukaemic cell is uncertain, but it is probably related to the reticulum cell or lymphocyte.

A normochronic, normocytic anemia is commonly associated with this condition but no other abnormality of erythrocyte form has been reported to occur, nor did we find any such change with the light microscope in blood films and bone marrow smears from our case, apart from an occasional burr cell. However, in ultrathin sections of bone marrow and peripheral blood cells examined with the electron microscope, a remarkable alteration of form was seen in some of the erythrocytes, reticulocytes, and normoblasts (Figures 1 and 2). The alterations in form are often quite complex so that no two cells look exactly alike, but a basic feature seems to be the production of numerous branched and nubranched cell processes which in ultrathin sections often appear club-shaped. Such processes arise either from small foci or more extensive areas of the cell surface. This may culminate in the production of most bizarre forms which bear little resemblance to the original shape of the red blood cell. This abnormality is quite different from any of the well known alterations of erythrocyte morphology such as acanthocytes[5], helmet cells or schistocytes[6], sickle cells or the discocyte to echinocyte transformation in the normal and pathological cell[7]. Further the above mentioned abnormalities are discernable with the light microscope, while the new alteration of morphology we are now reporting can be characterized only with the electron microscope.

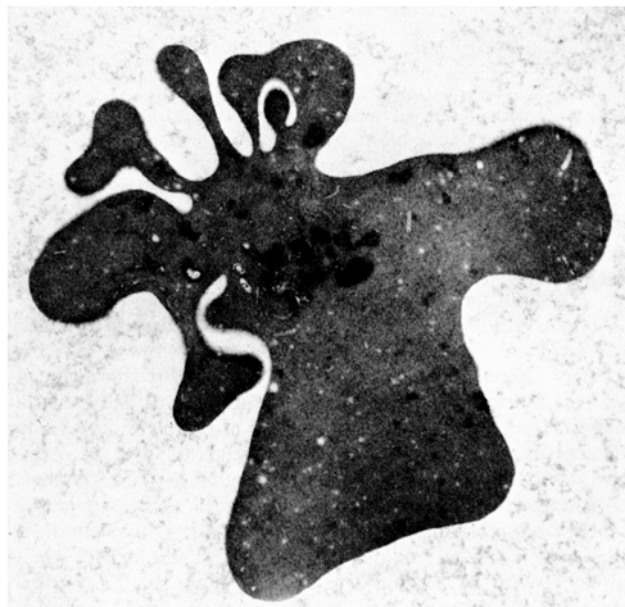

Fig. 1. Reticulocyte from peripheral blood showing alteration of overall shape and cell processes. ×10,000.