Immunology, 1967, **12**, 615.

Quantitative Analysis of the Chimæric State in Mice

II. CYTOLOGICAL EXAMINATION OF THE PROPORTION OF PROLIFERATING DONOR AND HOST CELLS IN RUNT DISEASE IN MICE

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(*Received 1st August* 1966)

Summary. The distribution of donor cells in the lymphoid organs of mice suffering from acute runt disease was investigated by means of the cytological marker (T6). In all the strain combinations studied relatively low proportions of donor cells were identified in the spleen and bone marrow. In the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations high proportions of donor cells were found in the lymph nodes and thymus. Few donor cells at all lymphoid sites were identified in the CBA-T6T6→C57BL combination, though the recipients showed unquestionable clinico-pathological signs of runt disease.

Despite marked splenomegaly in 12-day-old runts, the mean total number of cells in the spleen was similar to that in normal mice and mice injected at birth with isologous cells. In the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations donor cells significantly contributed to the total cellularity of the enlarged spleen, in contrast to the CBA-T6T6→C57BL combination in which the host's spleen consisted almost exclusively of host cells.

Grafting tests showed that runted mice can be either non-specifically or specifically tolerant which seemed to depend on the degree of lymphoid atrophy and the degree of chimerism. Some of the animals that eventually rejected skin grafts from spleen inoculum donors, continued to be chimeric for donor lymphoid cells.

A mechanism for runt disease could be mutual interaction between donor and host cell populations causing reduction of the host's lymphoid reserve and consequent appearance of clinical symptoms of disease.

INTRODUCTION

Runt disease will develop when immunologically competent cells are inoculated into genetically unrelated neonatal hosts. The condition has been ascribed to the immunological reaction of grafted donor cells against the host (Billingham and Brent, 1959). This hypothesis logically implied an appreciable contribution by donor cells to the lymphoid cell population of the host.

Several cytological analyses of the spleen in runt disease performed so far have shown that, contrary to the expectation, donor cells constituted only a fraction of all the dividing

cells. Thus, Davies and Doak (1960) performed the analysis of the chromosome constitution of the cells in metaphase (T6) present in the spleens of mice suffering from runt disease. They reported that donor cells were virtually absent from the spleens of the sick animals and concluded that splenomegaly in young mice injected at birth with homologous adult spleen cells was not primarily due to the proliferation of donor cells. In a similar experiment, Nakić, Kaštelan, Bunarević and Mitin (1965a) also observed a low frequency of donor cells in the spleen of acute runts, although donor cells were regularly found. Particularly relevant in this respect was the report by Nowell and Defendi (1964). These authors investigated the distribution of proliferating donor cells in runt disease in rats by means of sex chromosome differences. They also found that splenomegaly largely resulted from proliferation of host cells. However, lymph node enlargement was found to have resulted from donor cell proliferation. Thus, donor cells were present in large numbers in the lymph nodes, were much less common in the spleen, and rare in the thymus and bone marrow.

During the study of distribution of donor cells in the lymphoid organs of mice neonatally inoculated with *isologous* spleen cells, Nakić *et al.* (1965b) and Nakić and Kaštelan (1967), found appreciable proportions of isologous cells in the lymph nodes, but very low proportions in the spleen, thymus and bone marrow.

These results raised the question whether in mice neonatally inoculated with *homologous*, i.e. genetically foreign spleen cells, a similar distribution of donor cells would be found, i.e. selective proliferation of donor-type cells in the lymph nodes, with low proportions in the spleen, thymus and bone marrow. It was also important to establish whether—as suggested by Nakić (1962) and by Nowell and Defendi (1964)—a high incidence of proliferating donor cells in the lymph nodes were a necessary prerequisite for the development of runt disease irrespective of the strain combination used.

The work recorded here gives full details of the experiments designed to answer the following questions:

(1) What is the proportion of dividing donor cells in the spleen of mice at the time when clinical symptoms of runting are fully developed?

(2) Are similar results obtained if different strain combinations are used?

(3) What is the proportion of donor cells in the spleen in the period preceding the appearance of clinical symptoms of runting?

(4) What is the pattern of distribution of donor cells in the spleen, lymph nodes, thymus and bone marrow at the height of splenomegaly?

(5) Is splenomegaly due to the proliferation of donor cells, host cells or both?

(6) Are 'runted' mice tolerant, and if so, are they specifically tolerant or not?

MATERIALS AND METHODS

Mice of highly inbred strains: A/H, CBA/H-T6T6, CBA/H and C57BL/H were used in these experiments. The strain CBA/H-T6T6 is histocompatible with strain CBA/H (Barnes, Loutit and Micklem, 1962).

In all the experiments spleen cell suspensions from adult homologous donors were inoculated intraperitoneally into newly born mice within 12 hours after birth. The dosages of viable cells injected per host ranged from 20 million to 100 million. These relatively high dosages were used because of the reported lower efficacy of the intraperitoneal route of injection as compared with the more commonly employed intravenous route (Billing-

ham and Brent, 1959). The method of preparing lymphoid organs for cytological analysis has been previously described (Nakić, Teplitz and Ohno, 1966). Donor and host cells were identified by the presence or absence of the characteristic T6 chromosomes.

The proportion of proliferating donor cells in the spleen of mice displaying advanced symptoms of runt disease was studied in four donor–host combinations: A→CBA-T6T6, CBA-T6T6→A, C57BL→CBA-T6T6 and CBA-T6T6→C57BL. The recipients were killed for analysis on the day when judged to be *in extremis*. Clinical diagnosis of runt disease was supported in all cases by histological analysis.

The proliferative activity of donor cells in the spleen in the period before the appearance of clinical symptoms of runt disease was examined in the A→CBA-T6T6 combination. The recipients were killed and analysed cytologically 3, 4 and 7 days after birth.

The proportion of dividing donor cells in the lymph nodes (inguinal, axillary and submandibular), thymus and bone marrow in animals suffering from acute runt disease was studied in three strain combinations: A→CBA-T6T6, C57BL→CBA-T6T6 and CBA-T6T6 →C57BL. For comparison, the spleen was analysed simultaneously with other lymphoid organs. All the animals were killed, and analysed cytologically and histologically 12 days after birth.

The weight and cellularity of the spleen both in normal, untreated neonatal mice and mice neonatally injected with isologous (CBA→CBA-T6T6 and vice versa) or homologous spleen cells (A→CBA-T6T6 or C57BL→CBA-T6T6) was determined on the day of birth and on days 3 and 12 after birth. The procedure for determining the cellularity of the spleen (total number of expressible cells from spleen) has been described elsewhere (Nakić and Kaštelan, 1967).

Tolerance of skin homografts in 'runts' was tested by challenging the hosts with skin grafts from spleen inoculum donors and donors unrelated to either inoculum donor or recipient. The technique of skin grafting was that used in previous experiments (Nakić and Silobrčić, 1962). The animals were also analysed cytologically and histologically.

RESULTS

Table 1 records results of the cytological analysis of the spleens of mice suffering from acute runt disease. As can be seen, donor cells were regularly found in all the four strain combinations, but the proportion of donor-type cells was low in the great majority of animals. The lowest values were found in the CBA-T6T6→C57BL combination. Approximately two-thirds of runts contained in their spleens less than 20 per cent of donor cells and as low as 3 per cent. Only two out of twenty-eight animals analysed contained roughly equal proportions of donor and host cells. Following the inoculation of comparable numbers of spleen cells, the animals died earlier in the CBA-T6T6→A, than in the reverse combination. It was for this reason that the A→CBA-T6T6 combination was used in the next experiment.

In order to find out whether the proportion of donor cells in potential runts might initially be higher and then drop off as the clinical symptoms develop, some of the CBA-T6T6 mice neonatally inoculated with A spleen cells were killed and analysed cytologically during the 1st week of life, i.e. before the full blown symptoms of runt disease became manifest.

The analyses were performed 3, 4 and 7 days after birth. The results (Table 2) show that in the A→CBA-T6T6 combination donor cells during the 1st week of life are present

TABLE 1

CYTOLOGICAL ANALYSIS OF THE SPLEENS OF MICE SUFFERING FROM 'RUNT DISEASE'*

Donor	Host	Dosage of spleen cells per host (in millions)	Age of host at time of killing (days after birth)	Donor cells/ total mitotic figures
CBA-T6T6	C57BL	60	7	4/84
		60	7	2/56
		60	18	3/95
		60	18	0/49
		60	28	4/60
		60	28	0/7
CBA-T6T6	A	30	8	2/11
		30	8	11/69
		35	11	1/20
		100	12	1/15
		60	14	5/115
		40	14	1/8
		40	14	11/60
		20	15	2/52
		30	20	32/131
		40	20	3/52
C57BL	CBA-T6T6	70	14	7/17
		70	19	0/5
		70	19	74/134
		70	19	10/106
A	CBA-T6T6	25	16	20/55
		50	17	5/19
		30	18	7/86
		50	18	2/18
		30	19	15/80
		25	19	3/86
		20	27	4/117
		25	30	4/28

* All the hosts injected intraperitoneally with homologous spleen cells within 12 hours of birth.

TABLE 2

CYTOLOGICAL ANALYSIS DURING THE FIRST WEEK OF LIFE OF CBA-T6T6 MICE NEONATALLY INJECTED WITH A SPLEEN CELLS: SPLEEN CHIMERISM BEFORE DEVELOPMENT OF THE SYMPTOMS OF 'RUNT DISEASE'*

Dosage of A spleen cells per host (in millions)	Age of CBA-T6T6 at time of killing (days after (birth)	Donor cells/ total mitotic figures
40	3	5/69
40	3	4/62
60	4	0/10
40	7	10/46
60	7	11/141
60	7	4/112

* All the hosts injected intraperitoneally with homologous spleen cells within 12 hours of birth.

in recipient's spleen in proportions similar to those found later on when clinical symptoms of runt disease are fully developed.

The distribution of dividing donor cells in the lymph nodes, thymus and bone marrow of mice suffering from acute runt disease was studied in the A→CBA-T6T6, C57BL→CBA-T6T6 and CBA-T6T6→C57BL combinations. Since in previous experiments clinical and pathological signs of disease were well evident by the 10th to 14th day post natal in the majority of animals, day 12 post natal was selected as the day on which the hosts in this and in the next experiment would be killed and cytological analysis performed from the lymph nodes, thymus and bone marrow. For comparison, simultaneous cytological study of the spleen was also made.

TABLE 3

A CYTOLOGICAL ANALYSIS OF LYMPHOPOIETIC ORGANS OF MICE DISPLAYING ACUTE SYMPTOMS OF 'RUNT DISEASE'*

Strain combination	Dosage of spleen cells per host (10^6)	Donor cells/total mitotic figures			
		Spleen	Lymph nodes	Thymus	Bone marrow
A→CBA-T6T6	30	4/34	195/207	16/35	
	50	1/31	10/115		
	50	0/56	11/13		
	30	19/63	99/104	14/47	0/16
C57BL→CBA-T6T6	20	2/11	15/15	5/6	
	30		1/10		2/34
	30	4/72			18/100
	30	11/88	12/47		33/211
	30	4/70			18/130
	20	7/58	6/6	6/8	
	20	12/60	2/29	0/4	
	20	0/16	18/19	1/6	
CBA-T6T6→C57BL	50	1/102	0/18	0/10	0/23
	50	1/55	5/125	0/35	
	50	0/88	0/46	0/35	0/156
	20	0/63	5/75	0/53	0/36
	50	3/150	4/100	2/100	
	20	0/73	4/101	0/32	2/109
	20	0/102	10/208	0/116	1/111

* All the hosts injected intraperitoneally with adult donor spleen cells within 12 hours of birth. All the animals were killed and analysed cytologically on the 12th day of life.

Results are presented in Table 3. Cytological data for the spleen were comparable to those obtained in previous experiments (cf. Tables 1 and 2). In contrast to the spleen, the lymph nodes were found to be the site of most vigorous proliferation of inoculated cells. In the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations the proportion of dividing donor cells was high and in six out of ten animals examined few host cells were found. In these animals high-degree chimærism in the lymph nodes was associated with high levels of donor cells in the thymus. The frequency of donor cells in the bone marrow was much lower than that in the lymph nodes and thymus, and was similar to that found in the spleen.

In striking contrast to the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations, few donor cells were identified in the CBA-T6T6→C57BL combination at all sites, although the recipients showed unquestionable clinical and pathological signs of acute runt disease.

Table 4 gives data on the weight and cellularity of the spleen determined within 12 hours after birth and again on days 3 and 12 post natal in CBA-T6T6 mice either normal or neonatally inoculated with 20–60 million isologous (CBA) or homologous (A or C57BL) spleen cells.

The mean value (±S.E.) for the weight of the spleen in twenty-one neonatal mice was 1·79±0·28 mg (range: 0·8–4·1 mg). Normal untreated 3-day-old mice had the mean spleen weight of 5·4±0·41 mg (3·6–8·3 mg). No significant difference ($P>0·10$) in the mean spleen weight was found in 3-day-old mice injected at birth with 45–60 million isologous adult spleen cells (4·6±0·59 mg; 2·0–8·0 mg). In normal untreated 12-day-old animals the mean weight was 19·1±0·94 mg (15·2–23·0 mg). Some spleen enlargement

TABLE 4

SPLEEN WEIGHT AND CELLULARITY IN NORMAL MICE AND MICE* NEONATALLY INJECTED WITH ISOLOGOUS OR HOMOLOGOUS SPLEEN CELLS†

Treatment of host	No. mice	Age at time of killing (days after birth)	Spleen weight (mean values ± S.E. in mg)‡	Spleen cellularity (mean value ± S.E. in millions)
—	21	0	1·79±0·28 (0·8–4·1)	1·93±0·31 (0·75–3·92)
—	12	3	5·4±0·41 (3·6–8·3)	6·2±0·51 (3·9–9·6)
$45–60\times10^6$ isologous spleen cells	10	3	4·6±0·59 (2·0–8·0)	5·1±0·9 (1·7–11·8)
—	7	12	19·1±0·94 (15·2–23·0)	44·1±3.3 (29·2–57·0)
$20–30\times10^6$ isologous spleen cells	6	12	25·05±1·01 (19·9–28·0)	36·6±3·5 (26·0–47·2)
$20–30\times10^6$ homologous spleen cells	13	12	39·1±2·8 (27·3–58·2)	42·0±5·2 (21·3–75·8)

* All mice belonged to CBA or CBA-T6T6 strain. Animals that were inoculated received an intraperitoneal injection of isologous or homologous adult spleen cells within 12 hours after birth (see text).

† Includes data given elsewhere (Nakić and Kaštelan, 1967).

‡ Range in parentheses.

was found in 12-day-old mice inoculated at birth with 20–30 million adult isologous spleen cells, the mean spleen weight being 25·05±1·01 mg (19·9–28·0 mg). The difference in respect to normal animals of the same age was found to be statistically highly significant ($P<0·001$). Marked splenomegaly resulted from neonatal inoculation of 20–30 million adult A or C57BL spleen cells, the mean spleen weight on the 12th day post natal being 39·1±2·8 mg (27·3–58·2) mg. The difference in weight is highly significant both in respect to normal mice and mice inoculated at birth with isologous cells ($P<0·001$).

The cellularity of the spleen (total number of expressible cells from the spleen) in normal neonatal mice was found to be 1·93±0·31 million (range: 0·75–3·92 million), rising in normal 3-day-old animals to 6·2±0·51 million (3·9–9·6 million) and in normal 12-day-old mice to 44·1±3·3 million (29·2–57·0 million). Mean cellularity in 3-day-old mice neonatally inoculated with isologous cells was 5·1±0·9 million (1·7–11·8 million) and in 12-day-old mice 36·6±3·5 million (26·0–47·2 million), neither value being statistically different from that in normal animals ($P>0·10$ and $P>0·05$ respectively). The

mean cellularity for the spleen in 12-day-old mice suffering from acute runt disease was 42·0±5·2 million (21·3–75·8 million). Despite marked splenomegaly this value was not statistically different from that found in normal mice and mice neonatally treated with isologous spleen cells ($P>0.1$).

If the cellularity of the spleen is expressed as millions of cells per milligram of spleen tissue, then this ratio is approximately 1·1 in normal neonatal mice and also in both groups of 3-day-old mice. The ratio is 2·3 in normal 12-day-old animals, 1·5 in 12-day-old mice neonatally inoculated with isologous cells and 1·1 in 12-day-old runts.

Histologically, the spleens of 12-day-old runts revealed loss of normal structure. The number of small lymphocytes was greatly diminished and the remaining ones were

FIG. 1. Two CBA-T6T6 littermates 25 days after birth. The runted mouse had been injected at birth with 30×10^6 C57BL spleen cells. The larger mouse (untreated) weighs 11 g, the runt only 3 g. Note areas of alopecia on the head and back of the animal and stunted appearance.

widely spaced. The white pulp was replaced to a great extent by reticulum cells, blast cells and neutrophilic granulocytes. The spleens of mice neonatally inoculated with isologous spleen cells also showed a somewhat 'rarified' appearance, but the white pulp consisted of mature lymphocytes forming smaller follicle-like aggregates.

Some of the CBA-T6T6 runts neonatally injected with A spleen cells survived for more than 3 weeks and were tested for tolerance. Because of the technical difficulties involved and a very poor physical condition of the animals (Fig. 1), only few survived skin grafting procedures and are presented in Table 5. All the hosts received skin grafts from A (spleen inoculum donor) and C57BL mice (third party grafts). Animals Nos. 1 and 2 died with both grafts preserved before cytological analysis could be performed. At the time of death (52 and 64 days after birth) the body weight of these animals was 6·5 and 7·5 g respectively. Histological analysis revealed severe lymphoid atrophy and multiple necrotic foci in the liver. Animals Nos. 3–8 displayed varying degrees of specific tolerance. All, except mouse

No. 5, rejected C57BL graft in 11–13 days, while continuing to carry the A graft. The mean survival time of C57BL grafts was >16·3 days (normal mean survival time of skin grafts transplanted from A and C57BL donors to CBA-T6T6 recipients: A→CBA-T6T6, 13·0±0·6 (range: 10–16) days; C57BL→CBA-T6T6, 10·4±0·2 (9–12) days). Superficial scaling or small necrotic areas appeared in all A grafts between the 9th and 12th post-operative days. This caused lesser or greater degree of atrophy and contraction of the grafts which in mouse No. 3 led to complete rejection of the A graft on the 27th post-operative day. In mice Nos. 4–7, the process of rejection became seemingly arrested during the

TABLE 5

SKIN GRAFTING TESTS IN CBA-T6T6 MICE SUFFERING FROM CHRONIC 'RUNT DISEASE' FOLLOWING NEONATAL INOCULATION OF A SPLEEN CELLS*

Animal No.	Dosage of spleen cells per host (in millions)	Skin graft tests: First test grafts† Day of grafting (after birth)	First test grafts† A survival time (days)	First test grafts† C57BL survival time (days)	Second A graft Day of grafting (after birth)	Second A graft Survival time (days)	Age of host at death (days after birth)	Donor cells/total mitotic figures: Spleen	Lymph-nodes	Thymus	Bone marrow
1	40	33	>19	>19			52				
2	25	39	>25	>25			64				
3	25	39	27	13			67	3/41			
4	80	33	>35	12			68	31/134			
5	30	40	>31	26			71	6/161		1/28	6/226
6	50	25	51	13	58	18	76	3/113			
7	30	39	>61	11	71	>29	100	14/75			
8	40	38	55	11	69	>32	101	10/59	11/131	0/11	5/106

* All the hosts injected intraperitoneally with adult donor spleen cells within 12 hours of birth.
† Normal mean survival time of skin grafts transplanted from A and C57BL donors to CBA-T6T6 recipients: A→CBA-T6T6, 13·0±0·6 (range: 10–16) days; C57BL→CBA-T6T6, 10·4±0·2 (9–12) days.

3rd post-operative week with subsequent recovery of the graft as shown by the re-appearance of normal skin texture, increase in graft size and growth of hair. Mouse No. 5, which had rejected C57BL graft in 26 days, had been in very poor condition throughout with intermittent diarrhoea. At the time of death (71 days after birth) its body weight was 6 g, compared with 20–22 g in controls of the same age, strain and sex. Cytological analysis revealed low proportions of donor cells in the spleen, thymus and bone marrow. In mouse No. 6 the A graft continued to survive in good condition until the host was challenged with the second A graft. The first test graft then began to deteriorate and both grafts broke down completely 18 days later. Although the clinical symptoms of runting at the time of killing were not pronounced in this animal, histological analysis showed atrophy of lymphoid organs and multiple necrotic foci in the liver. Despite relative immunological competence of mice Nos. 3 and 6 as shown by their capacity to eventually reject skin grafts from spleen inoculum donors, these animals continued to be chimæric for donor lymphoid cells in their spleens. Mouse No. 4 was killed 68 days after birth (35 days after grafting) carrying the A graft in good condition (Fig. 2). The body weight of the animal at the time of killing was 11·5 g. Mouse No. 7 received a second A graft 32 days following transplantation of the first test graft. Twelve days later, small necrotic areas appeared on the second A graft (Fig. 3) healing up 1 week later with subsequent

growth of dense hair (Fig. 4). During the immune crisis the first graft showed no changes except some thinning of hair. Cytological analysis of mice Nos. 4 and 7 showed that approximately 20 per cent of the dividing cells in their spleens were of donor origin.

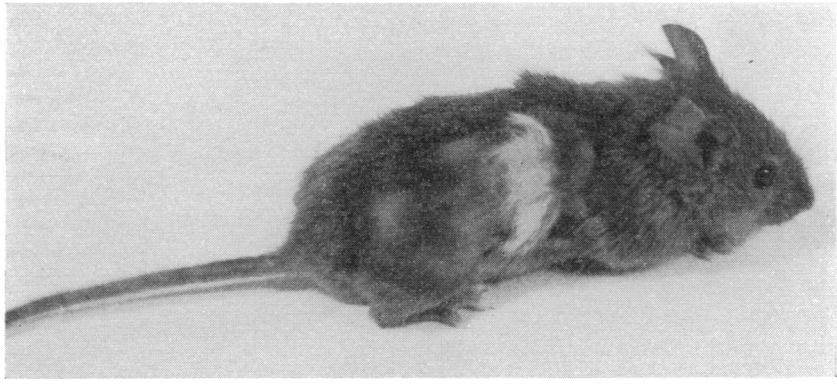

FIG. 2. CBA-T6T6 runt specifically tolerant of A skin graft, killed 68 days following neonatal inoculation of A spleen cells and 35 days after grafting. The weight of the animal at time of killing was 11·5 g.

FIG. 3. Specifically tolerant CBA-T6T6 mouse 46 days after first A graft (placed caudally) and 14 days after second A graft. Note small necrotic patches in the second A graft which appeared 11 days after grafting.

FIG. 4. Same animal as in Fig. 3 photographed 12 days later.

Mouse No. 8 rejected C57BL graft in 11 days and continued to carry an atrophic A graft which never grew hair. Following the transplantation of the second A graft on the 69th post-natal day, the first test graft was completely rejected 24 days later. The second A

graft also underwent atrophy and contraction. On the day of killing, 101 days after birth, the graft was very much reduced in size but growing hair. Despite the low degree tolerance of skin grafts, an appreciable proportion of donor cells was identified in the spleen, and donor cells were also found in the lymph nodes and bone marrow.

DISCUSSION

The results described in the present paper confirm the findings of Nowell and Defendi (1964) that the lymph nodes and not the spleen are the site of predilection for the proliferation of the injected foreign spleen cells. Although Nowell and Defendi have used a different species (rat), a different route of administration (intravenous) and a different cell inoculum (lymph nodes and buffy coat), from the species (mouse), route (intraperitoneal) and inoculum (spleen) used in the experiments described herewith, the results are essentially similar.

The results obtained with the *isologous* combinations (Nakić and Kaštelan, 1967) have provided us with a standard by comparison with which the relative strength of the graft *versus* host and host *versus* graft reactions can be measured. Following intraperitoneal inoculation of isologous spleen cells into neonatal mice almost 20 per cent of donor-type cells were found in the lymph nodes but only 2·5 per cent in the spleen, 1 per cent in the thymus and 0·3 per cent in the bone marrow. The results were strikingly uniform throughout the observation period for each lymphoid site. This showed that factors other than immunological have little influence upon the entry and the rate of proliferation of the injected spleen cells in the lymphoid organs of normal perinatal animals.

It seems reasonable to assume that shortly after the inoculation into neonatal hosts, *homologous* spleen cells are distributed in the lymphoid organs of the recipient in proportions similar to those found in the isologous combination. Should no competition between the two mutually incompatible cell populations occur, the frequency of homologous donor cells at any time following the inoculation would be expected to be similar to that in the isologous recipients. Such, for example, would be the case if donor and host cells acquired tolerance of each other. It was shown, however, that the frequency of homologous donor cells in the lymphoid organs of mice suffering from acute runt disease not only lacked the uniformity but also showed marked deviations either above or below the standard set by the isologous combinations. The high degree of chimærism found in the lymph nodes and thymus of the runted animals in the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations might lead one to conclude, as indeed was done by Nowell and Defendi (1964) and as was earlier suggested by Nakić, Silobrcić, Nakić and Bunarević (1961) and Nakić (1962) that a rapid proliferation of donor cells inducing a high donor–host cell ratio is an essential prerequisite for the development of runt disease. However, a very low frequency of donor cells at all lymphoid sites in the CBA-T6T6→C57BL combination in animals displaying unquestionable signs of runting, shows that—at least in some strain combinations—runt disease can begin, develop and proceed to its fatal end with donor cells making up only a fraction of the total lymphoid cell population. A similar conclusion has been reached following chimæric analysis of another transplantation disease: parabiotic disease (Nakić *et al.*, 1966).

The results described in this paper indicate that both graft *versus* host and host *versus* graft reactions occur simultaneously in the perinatal mouse inoculated with homologous

spleen cells. In some strain combinations it is the former reaction that predominates inducing a high donor–host cell ratio in the lymph nodes and thymus. In other strain combinations host *versus* graft reaction is just sufficiently strong to inhibit the spreading tendency of donor cells and reduce their proportion below the level attained by isologous cells. As long as the competitive strength of the host is insufficient to eliminate donor cells completely, the mutual interaction between donor and host cells causes massive destruction of lymphoid tissue. The resulting reduction of the recipient's lymphoid reserve is then responsible for the appearance of clinical symptoms of runt disease (Barnes *et al.*, 1962).

Since in the present experiments we have used homologous strain combinations, the main emphasis as regards the nature of interaction between donor and host cell populations has been laid upon graft *versus* host and host *versus* graft *immune* reactions. However, there is increasing evidence that antigenically dissimilar cell populations can interact in ways not easily recognizable as immunological reactions. Target cells may be killed as a consequence of contact with incompatible surface structures; thus, semi-isologous F_1 hybrid lymphoid cells can kill the parental target cells efficiently (Möller, 1965). Hybrid F_1 mice of certain genetic constitutions may not support the growth of transplanted haematopoietic cells from one of the parental strain donors (Boyse, 1959; McCulloch and Till, 1963; Cudkowicz and Stimpfling, 1964). Fox (1962) employed chromosome marker technique to trace the fate of parental spleen cells injected into (CBA × C57BL) F_1 adult hosts and observed almost complete disappearance of CBA cells following an initial short-lived but tremendous burst of donor cell proliferation. This shows that phenomena which may not all be immunological can also interfere with the propagation of one or the other cell population.

It is not easy to explain why in animals suffering from runt disease the lymph nodes are the site of most vigorous proliferation of donor cells. A similar phenomenon has been observed in the isologous system; following neonatal inoculation of genetically compatible spleen cell suspensions the lymph nodes were also found to be the site of selective colonization by donor cells (Nakić and Kaštelan, 1967). Assuming a similar situation following injection of homologous cells, then a relatively high starting proportion in the lymph nodes would endow donor cells with a 'striking power' greater than that possessed by foreign cells at other lymphoid sites. Once a high-degree chimærism is induced in the lymph nodes of the runted animals, donor cells might be 'seeded' to other lymphoid organs. This would explain why higher proportions of homologous donor cells are found in the thymus, bone marrow and spleen than are found when isologous cells are used (Nakić *et al.*, 1965b; Nakić and Kaštelan, 1967).

Despite marked splenomegaly found in 12-day-old runts, the mean number of expressible cells from the spleen was not significantly different from that found in normal animals or those injected at birth with isologous spleen cells. The enlargement of the spleen is, therefore, due to a decrease in the number of cells per unit weight of spleen tissue. This is probably caused by the replacement of small lymphocytes by larger cell types (blasts) and by both non-specific and immune inflammatory oedema consecutive upon the interaction between donor and host cell populations. Similarly, the enlargement of the spleen in mice neonatally inoculated with isologous spleen cells might be a result of non-specific oedema accompanying increased destruction of cells. An important distinction, however, must be drawn between runts and mice inoculated with isologous cells. While the white and red pulp of the spleen in the latter animals is populated with mature

lymphocytes, these are scarce in mice suffering from runt disease and are replaced by blast cells, histiocytes and neutrophils. The normal number of cells present in the spleens of 12-day-old runts, together with a high mitotic rate and the disappearance of small lymphocytes indicates the existence of a process responsible for the destruction of cells in excessive numbers.

The analysis performed in the isologous combinations (CBA→CBA-T6T6 and vice versa) has revealed that only some 50,000 cells out of 20–100 million injected into newborn mice actually enter the spleen (Nakić and Kaštelan, 1957). The number of isologous donor cells rises in 12 days to approximately 1 million (2·6 per cent out of 44 million). Since in the majority of 12-day-old runts in the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations the percentages of donor cells were between 10 and 30 per cent, the number of homologous donor cells must have risen from the initial 50,000 to something between 4 and 12 million (10–30 per cent out of 42 million). That means that each cell has had to divide at least six to eight times in 12 days even if immune destruction of cells is not taken into account. Consequently, in the A→CBA-T6T6 and C57BL→CBA-T6T6 combinations the proliferation of donor cells represents a significant factor in the maintenance of the 'normal' number of cells in 12-day-old runts. The situation is different in the CBA-T6T6→C57BL combination where the frequency of donor cells was found to be below the level found in the isologous combinations.

Grafting tests performed in runts give some answer to the question whether animals suffering from runt disease are tolerant or not. It was found that mice suffering from runt disease can be either non-specifically or specifically tolerant apparently depending upon the degree of lymphoid atrophy and the degree of chimærism.

The presence of donor cells in the lymphoid organs of animals that eventually succeeded in rejecting skin grafts from spleen inoculum donors merits some mention. The phenomenon has been already described in rat parabionts (Nakić, Kaštelan and Avdalović, 1962) and mouse parabionts (Nakić, *et al.* 1966) and might be another example of the so called 'split' tolerance described in mice (Brent and Courtenay 1962; Silobrčić, 1965), mice and rats (Billingham and Silvers, 1962) and chicks (Štark, Krěn, Frenzl and Brdička, 1962) rendered tolerant by neonatal inoculation of homologous or F_1 hybrid cells. In mouse parabionts (Nakić *et al.*, 1966) and in mice rendered tolerant by neonatal inoculation of F_1 hybrid cells (unpublished data) the phenomenon was found to be associated with low-degree chimærism.

ACKNOWLEDGMENTS

This work was supported by Research Grants Nos. 87 from KOMNIS (Belgrade), Nos. 3932/1 and 814/2 from the Federal Scientific Fund (Belgrade) and No. 32/R from the Scientific Fund of S.R. of Croatia (Zagreb).

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