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Induction of Runt Disease in Adult Mice by Pre-sensitized Homologous Lymphoid Cells.* (27407)

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The condition of runt disease has now been generally accepted as an immunologic reaction of graft against host tissue(1). This syndrome, often referred to as either homologous, wasting or secondary disease, has been described as the consequence of introducing immunologically competent adult cells into host animals incapable of rejecting the donor cells. The unresponsive hosts have been either fetal or neonatal animals, or adult animals which have been made tolerant at birth or have been subjected to lethal irradiation. Another form of immunological unresponsiveness has been seen in F_1 hybrids which were genetically incapable of rejecting their parental tissues.

Injection of homologous lymphoid cells into immunologically competent adults has not usually resulted in runt disease presumably because of the rapid destruction of the injected cells by the host. Castermans(2), however, in an attempt to produce Felton's type of immune paralysis to tissue antigens, used repeated injections of homologous splenic cells and was able to show a partial abrogation of the host's immune response to homografted skin. In addition, he observed lymphoid hypoplasia, loss of body weight and other characteristics of runt disease in some of the injected animals. More recently, Shapiro *et al.*(3) were able to achieve in parental animals "tissue tolerance" to their F_1 hybrids after repeated injections of F_1 splenic cells.

The purpose of this study was to investigate the possibility of inducing runt disease in normal adult mice with a single injection of homologous lymphoid cells from donors sensitized to the prospective host's tissues. It was also desired to learn whether, by injection of a proper number of homologous cells, a nice balance could be achieved between host and foreign cells so that a permanent chimera might be created without the complication of runt disease.

Materials and methods. An outline of the method is illustrated in Fig. 1. Two inbred strains of mice, A and CBA, were used throughout the study. Seventy-five mice of each strain were sensitized to the homologous strain by cross skin grafts. To harvest sensitized cells from regional cervical and axillary lymph nodes, the skin grafts were cut into triangles and placed on recipients with the apex of the graft in the nuchal region and the base crossing the scapulae. To insure a maximal sensitization, all the animals were injected intraperitoneally with 10^7 homolo-

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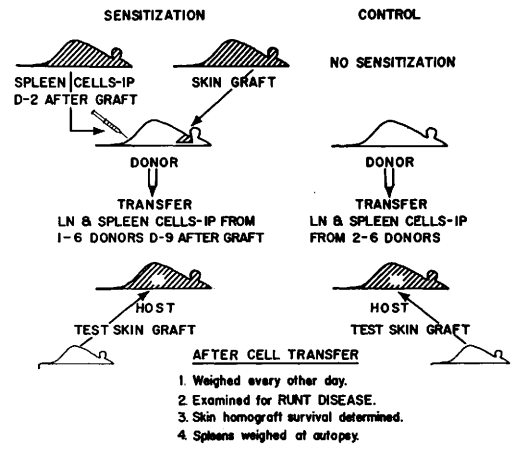

FIG. 1. Experimental model for induction of runt disease in adult mice by pre-sensitized homologous lymphoid cells.

gous spleen cells on the second day after skin grafting. Both groups of animals were then sacrificed on the 9th day after skin grafting.

Cell suspensions were prepared by shredding the axillary and cervical lymph nodes and spleens with rakes in balanced salt solution (Hanks') containing 20% PVP by volume. Aliquots of this suspension were taken for cell counts in the hemocytometer and for estimation of cell viability by the trypan blue staining technic. The cell suspensions from each strain, along with the residual cellular brei, were then injected intraperitoneally into 6 groups each consisting of 3 normal adult mice of the opposite strain. Preparation and transfer of the cell suspensions were done under careful aseptic conditions. Recipients were grouped according to the number of lymphoid equivalents§ administered. The lymphoid equivalents injected ranged from one through six. An additional group of 12 control animals of each strain received lymph node and spleen cell suspensions from non-sensitized isologous donors prepared in the same manner as for the experimental groups. The controls were divided into 3 groups of 4 mice and each group received 2, 4, or 6 lymphoid equivalents. All animals were grafted with homologous skin of the opposite strain within 24 hours of the cell transfer.

Body weights of the animals were recorded every other day and skin grafts inspected daily for survival and checked by periodic biopsy. To minimize post-mortem autolysis, the animals were checked 3 times daily and sacrificed if they appeared obviously moribund. At time of death or sacrifice, tissues were inspected grossly and weights of the spleens recorded. Lymph nodes and spleens were prepared for histology and stained with hematoxylin and eosin.

A comparative spleen index was calculated by dividing the absolute weight of the spleen in milligrams by the weight of the mouse in grams. To arrive at a normal spleen index for CBA and A mice in our colony, 30 normal mice of each strain were sacrificed and a spleen index of 3.5 ± 0.2 mg/g body weight and 4.1 ± 0.2 mg/g body weight was determined for CBA and A mice, respectively.

After 40 days, all the surviving animals had sloughed their homologous skin grafts and were regrafted 5 days later with homologous skin to determine the time of second set response. The remaining animals, which survived for an additional 17 days, were sacrificed to terminate the experiment 62 days after cell transfer.

Results. Pre-sensitized lymphoid cells when injected into homologous hosts produced the clinical picture of runt disease, *i.e.*, weight loss, general lethargy, hunched posture, ruffled fur, scaly conjunctivitis and blindness.

In A mice recipients of CBA pre-sensitized cells, the full development of the disease was most readily recognizable (Table I). Seven of 12 mice which had received from 3 to 6 lymphoid equivalents showed weight losses significantly greater than controls receiving 4 to 6 lymphoid equivalents of non-sensitized cells. Mice which had been injected with one or 2 lymphoid equivalents of sensitized cells exhibited no greater weight losses than controls receiving similar numbers of non-sensitized cells. In those experimental animals showing significant loss of body weight, the decrease was progressive and gradual,

§ A lymphoid equivalent is the number of regional lymph node and spleen cells obtained from a single donor mouse, and in this experiment, ranged between 180 and 260 $\times 10^6$ lymphoid cells from sensitized mice and between 120 and 200 $\times 10^6$ lymphoid cells from non-sensitized mice.

TABLE I. Summary of Data on A Mice Injected with Sensitized and Non-Sensitized CBA Lymphoid Cells.

Recipient mice		No. of lymphoid equivalents*	Maximum wt	Day of maximum	Day of death or	Spleen index (mg spleen/	Survival time of skin
No.	Wt (g)	transferred	loss (g)	wt loss	sacrifice	g b.w.)	homograft
1	24.4	6	4.4	9	34	1.5	34
2	21.6	6	.7	2	2	2.7	2
3	20.1	6	.3	7	62	5.1	32
4	20.3	5	6.7	20	62	4.5	38
5	23.3	5	2.7	13	62	7.6	31
6	20.0	5	1.8	11	18	8.7	18
7	20.7	4	.2	7	62	3.8	26
8	20.2	4	5.0	27	27	2.6	20
9	24.3	4	1.9	3	62	4.5	23
10	22.9	3	1.4	10	62	3.1	29
11	20.0	3	.4	4	30	4.0	18
12	21.5	3	.3	11	62	6.7	22
13	21.6	2	.7	3	62	3.6	14
14	20.3	2	.3	6	35	8.3	19
15	23.6	2	.2	1	62	3.4	16
16	20.0	1	0	—	62	2.7	20
17	21.4	1	0	—	62	3.5	17
18	21.8	1	0	—	62	5.1	14
Controls			Transfer of Non-Sensitized CBA Lymphoid Cells				
21	20.0	6	.7	4	62	3.9	14
22	18.5	6	.4	2	62	4.5	17
23	21.3	6	.2	1	62	3.7	12
24	22.4	6	.9	7	62	4.5	17
25	23.1	4	.1	5	62	4.1	15
26	21.0	4	0	—	62	4.4	13
27	20.9	4	.3	3	62	3.6	15
28	21.7	4	.3	10	62	4.8	12
29	24.1	2	.4	2	62	3.6	14
30	23.2	2	.1	1	62	4.0	15
31	20.2	2	.2	1	62	4.4	12
32	21.1	2	0	—	62	3.9	12

* Lymphoid equivalent is the number of regional LN and spleen cells obtained from a single mouse and ranged between 180-260 $\times$ 10^6 lymphoid cells from sensitized mice and between 120-200 $\times$ 10^6 lymphoid cells from non-sensitized mice.

reaching a maximum about 10 days after transfusion of cells; in controls, maximum weight loss occurred on the average 3 days after cell injection. At 3 weeks, the weight of all surviving experimental mice had returned to preinjection levels.

Survival time of CBA skin grafts on A hosts transfused with CBA pre-sensitized cells was increased significantly beyond the survival time of first set grafts in control A mice. The test grafts survived longest in hosts which had received the largest numbers of cells, and even in mice which had received one to 2 lymphoid equivalents, they survived longer by several days than the grafts applied to control A mice (Table I).

CBA recipients of pre-sensitized A cells were more severely affected than A mice receiving CBA cells. All animals transfused with 5 or 6 lymphoid equivalents died on the second or third day and showed marked weight loss (Table II). CBA mice which had received 4 lymphoid equivalents or less survived for varying periods of time after transfusion but only one remained to the end of the observation period. Weight loss was progressive and reached an average maximum of almost 6 g, 14 days after cell transfer. In control CBA's, recipients of non-sensitized cells, maximum weight loss was 1.6 g, attained within 6 to 7 days after injection.

A skin grafts on the experimental CBA hosts were retained until death without evi-

TABLE II. Summary of Data on CBA Mice Injected with Sensitized and Non-Sensitized A Lymphoid Cells.

Recipient mice		No. of lymphoid equivalents* transferred	Maximum wt loss (g)	Day of maximum wt loss	Day of death or sacrifice	Spleen index (mg spleen/ g b.w.)	Survival time of skin homograft
No.	Wt (g)						
1	30.0	6	5.0	3	3	2.7	3
2	30.1	6	2.7	2	2	1.6	2
3	29.4	6	2.6	2	2	2.2	2
4	29.5	5	3.4	2	2	1.9	2
5	31.9	5	3.2	2	2	2.2	2
6	29.7	5	5.5	3	3	2.2	3
7	29.5	4	7.4	5	5	5.7	5
8	30.1	4	4.6	18	18	2.7	18
9	30.6	4	6.1	25	25	2.3	25
10	31.0	3	4.0	22	22	3.5	22
11	28.9	3	8.3	7	7	5.0	7
12	31.9	3	6.7	6	27	14.8	27
13	30.0	2	2.1	5	5	3.6	5
14	25.9	2	4.4	12	21	9.6	21
15	30.0	2	3.6	11	11	2.2	11
16	28.1	1	5.7	7	62	3.6	34
17	29.2	1	6.0	12	36	11.0	36
18	26.0	1	5.7	37	37	6.2	37
Controls			Transfer of Non-Sensitized A Lymphoid Cells				
20	30.0	6	1.4	7	62	3.3	15
21	26.2	6	1.2	5	62	3.0	14
22	25.6	6	1.6	4	62	3.1	12
23	28.4	6	1.4	7	62	3.7	16
24	29.8	4	.8	8	62	3.4	12
25	30.1	4	1.2	6	62	3.9	11
26	27.4	4	1.1	9	62	4.3	13
27	28.6	4	1.6	5	62	3.3	12
28	31.0	2	.8	4	62	3.6	12
29	27.2	2	.5	9	62	4.1	11
30	28.8	2	.7	7	62	4.2	13
31	29.2	2	1.0	4	62	3.0	12

* Lymphoid equivalent is the number of regional LN and spleen cells obtained from a single mouse and ranged between 180-260 $\times$ 10^6 lymphoid cells from sensitized mice and between 120-200 $\times$ 10^6 lymphoid cells from non-sensitized mice.

dence of rejection. The one animal that survived 62 days rejected its test graft 34 days after placement. Of the 8 animals that lived beyond 11 days following cell transfusion, all retained their grafts well beyond the rejection time of 13 days recorded for survival of A skin grafts in control CBA hosts.

A systematic macro- and microscopic study of the spleen and lymph nodes was not made since the time and number of examinations depended upon the random deaths of the mice up to 62 days after transfer. There were, however, sufficient observations throughout the experimental period to indicate a biphasic pattern of changes in spleens and lymph nodes, particularly obvious in the CBA mice. During the first 2 to 3 days in CBA animals injected with 5 or 6 lymphoid equivalents, there was marked weight loss of the lymphoid tissues. At autopsy, the lymph nodes were found with great difficulty and spleen indices decreased 25-50% below the control spleen index (1.6-2.7 mg/g). Histologically, at this time, both the red and white pulp were cell "poor". The red pulp was congested with red cells, or was hemorrhagic, and the follicles were small and loose. The lymph nodes had the appearance of being flushed out. In the 12 CBA mice which had received 4 or less lymphoid equivalents, atrophy of lymphoid organs in 3 of these was observed 11-25 days after cell transfusion and

was similar histologically to the changes described above. Enlargement of lymphoid tissues occurred in 6 of the 12 and was characterized microscopically by proliferation of large basophilic mononuclear cells, probably immature lymphoid elements, massed together in clusters or broad sheets in the inter follicular area. In lymph nodes, numerous slightly eosinophilic large cells were aggregated in sinusoids. At 62 days after cell transfer in the one surviving CBA mouse, spleen and lymph node histology were indistinguishable from controls and normals.

In A mice, the pattern of spleen change was not so readily recognized as in CBA hosts. Of 6 A recipients that died during the experimental period, 3 showed decreased spleen indices, 2 showed elevated spleen indices and one had a spleen index within the normal range. Of the 12 animals that survived the 62 days, 5 exhibited decreased spleen indices and 4 exhibited lymphoid enlargement. The remaining 3 had spleens and lymph nodes within the normal range.

Discussion. This study has demonstrated that runt disease can be achieved in normal adult mice by transfer of homologous lymphoid cells which have been sensitized against the tissues of the prospective host. All the clinical and pathologic consequences of this condition that have been described in immunologically incompetent hosts injected with adult lymphoid cells were seen in these normal adult mice. The mechanisms operating at the beginning might be best explained as a classic graft *vs* host reaction. In the previously described conditions that resulted in runting, the host was incapable of rejecting the donor cells, but in our experiment the hosts were immunologically competent. The success of this laboratory model therefore lay in the advantage given to the donor cells which were capable of more rapid rejection of the host cells since the donor cells were previously sensitized and could react in an anamnestic or second set manner. The maintenance of the advantage initially held by the donor cells depended on the ability of the host to recover its immune mechanisms. If the host failed in this effort, death ensued, or if, on the other hand, the host recovered from the initial encounter, the donor cells were overthrown by sheer force of numbers. Howard *et al.*(4) have recently demonstrated that there was proliferation of host lymphoid cells in the presence of transferred donor cells. The additional advantage of pre-sensitization was further augmented by increasing the number of lymphoid cells transferred.

The finding of extended skin graft survival in the runted animals was consistent in both groups of mice. The mechanisms involved were not fully apparent but 2 possible explanations have been considered. The first was that the host's immunologic mechanisms had been temporarily destroyed or inactivated to such a degree by the donor cells that the host was unable to react to foreign tissues with a normal transplantation rejection. This type of abrogation of the immune response has been seen also in other stressful situations such as following whole body irradiation, administration of cortisone, uremia, etc. A second possible mechanism for extended skin graft survival has been related to a persistence of the cellular elements that have been transferred and resultant neutralization of the cellular or "humoral" host response, thus allowing the graft to enjoy a temporary residence without homograft rejection.

The final question raised by this study was whether or not it would be possible to find a dose of donor cells that would result in adult chimerism without the sequelae of runt disease. To investigate this possibility, graded doses of cells from one to 6 lymphoid equivalents were transferred to host mice. Our results indicated that either the donor cells would dominate and result in progressive runting and death or that the host would recover and reject the transferred cells. Since the graded doses were administered in increments of 180 to 260 $\times$ 10^6 cells per mouse equivalent, finding the balanced state of chimerism in normal animals might have been attained by transferring fewer cells in each dose increment. We feel, however, that this goal is unlikely and that in this biologic system the donor or host will eventually dominate.

Summary. The passive transfer of large numbers of homologous lymphoid cells pre-

sensitized to recipient tissues into normal adult mice was capable of inducing runt disease. The recipient mice showed the characteristic clinical and pathologic sequelae described in this condition when induced in immunologically incompetent hosts. Severity of the disease was influenced by number and strain of transferred cells. A strain cells elicited a more intense clinical and pathological reaction against their CBA hosts than was seen in the A host transfused with CBA cells. During the period of runting, homologous skin grafts of the opposite strain were tolerated far beyond the usual rejection time of untreated homografted A and CBA mice.

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Studies on the 5′ Adenylic Acid Deaminase of the Myofibrills.* (27408)

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The enzyme 5′ adenylic acid deaminase, first discovered in muscle by Schmidt(1), occurs in high concentration in this tissue in close association with myosin though its biological function is unknown. The enzyme has been purified in various ways and extensive studies of muscle 5′ adenylic acid deaminase have been published(2,3,4). Since there is some disagreement in results obtained with the "purified" enzymatic preparations, it was thought desirable to study some of the properties of this enzyme in the highly organized myofibrills. The myofibrillar suspension is readily prepared and while containing mainly actomyosin, also exhibits strong 5′ adenylic acid deaminase activity. Its use for the enzyme studies has the advantage of having the deaminase in a form less subject to modification from steps necessary for its chemical isolation and more akin to its possible structure in the intact muscle.

Materials and method. ATP, ADP, AMP 5′, AMP2′3′, GMP, IMP and ITP† were purchased from the Sigma Co., St. Louis, Mo. Plaquenyl hydroxychloroquine, atabrine and rivanol were kindly provided by Winthrop Laboratories, New York City. Quinidine sulfate was purchased from Merck & Co., Rahway, N. J. Camoquine dihydrochloride was a gift of Parke, Davis and Co. and proquanyl was donated by Ayerst Laboratories. Quinine hydrochloride was a product of Eli Lilly & Co. Acroflavin and acridine orange were products of National Aniline Co.

0.1 M succinate buffer was prepared from succinic acid and NaOH and 0.1 M citrate buffer from citric acid and NaOH.

The myofibrillar suspension was prepared in a 0.05 M, pH 7.0 borate buffer as previously described(5). The incubation mixture usually contained about 0.3 mg myofibrillar protein and 50.0 μM buffer in a 1.0 ml volume. The incubation was carried out at room temperature (23-25°) for 10 minutes and the reaction terminated by addition of 0.25 ml of 25% TCA solution. By means of controls run with each set of experiments it was found that changes due to temperature variation were negligible. Determination of the ammonia formed was performed with the Conway microdiffusion method(6) followed by Nesslerization.

Results. The maximal velocity of the reaction was obtained at 5.0 mM AMP con-

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† Abbreviations used: AMP = Adenylic acid (5′). AMP (2′3′) = A mixture of 2′ and 3′ adenylic acid. IMP = Inosinic acid (5′). GMP = Guanylic acid (5′). ADP = Adenosine diphosphate, ATP = Adenosine triphosphate, ITP = Inosine triphosphate.