Dissociated Thymus and Bone Marrow Cells: Synergism in Graft Versus Host Reaction

A Graft Versus Host (GVH) reaction occurs when immunocompetent cells capable of reacting against the host are inoculated into recipients unable to reject them. Bone marrow cells from adult donors are capable of producing a severe GVH reaction in lethally irradiated recipients 3 to 6 weeks after cell inoculation. Adult thymus cells, however, provoke only a poor GVH reaction when injected into histo-incompatible hosts[1–7].

In studies of humoral immunity it has been shown that an increased antibody response to sheep red cells results when adult thymus and bone marrow cells are allowed to interact in lethally irradiated adult hosts[8–14]. There have been further reports in which various experimental models with non-irradiated hosts have failed to show a synergism between thymus cells and bone marrow cells from adult donors in cell bound immune reactions[15–17].

The purpose of the present experiments was to determine whether thymus and bone marrow cells from newborn rats would be able to act synergistically during GVH reaction in lethally irradiated mice.

Materials and methods. Experimental animals: 12-week-old inbred male (C57 Bl/6 ♀ × DBA/2 ♂) BDF-1 mice were used as hosts. Cell donors were newborn rats of the inbred strain Bd IX[18]. Isologous control cells came from 6-week-old BDF-1 mice.

X-irradiation: The recipients received 850 rad of total body irradiation (220 KV; 13,9 mA; 55,18 r/min; HVL 1 mm Cu).

Cell suspensions: To obtain thymus cells the thymuses were cut in half and passed through an 80 mesh stainless steel sieve in cold BSS medium (Hanks) under aseptic conditions. The cells were centrifuged at a low speed and the sediment resuspended in BSS-medium. Viable cell counts were made with trypan blue. To isolate bone marrow cells scapula, humerus, femur and tibia were rinsed with 2°C sterile BSS-medium. The hosts were divided into 3 groups all of which received i.v. injections of either thymus cells (4×10^7 cells per animal), or bone marrow cells (4×10^7 cells per animal), or a combination of thymus and bone marrow cells (3×10^7 cells of each cell type per animal).

PFC assay. The spleens of chimeras were tested on hemolysin forming cells by the technique of JERNE et al.[19]. To distinguish between donor and host specific PFC, the spleen cells were prepared as described in earlier experiments[20].

GVH assay. The chimeras were examined on spleen enlargement, loss in body weight and anemia. The spleen index was calculated by the method of SIMONSEN et al.[21]. Microhematocrit determinations were made on blood samples taken from the retro orbital plexus.

Results. Hosts which were only inoculated with thymus cells died within the first 21 days after irradiation and cell inoculation. Symptoms due to a GVH reaction could not be identified in these hosts. Recipients of bone marrow cells and recipients of thymus and bone marrow cells which survived this interval were taken for further observation.

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Changes in spleen index and hematocrit values of lethally irradiated mice, inoculated with thymus (T), bone marrow (BM) or thymus and bone marrow (T + BM) cells from newborn rats

Days after irradiation and cell inoculation	Average spleen index[a] of chimeras (isologous substituted controls)			Mean hematocrit values[c] (%) of chimeras/ isologous substituted controls		
	T	BM	T + BM	T	BM	T + BM
10	0,79/0,82	0,81/0,89	0,83/0,86	38/35	37/39	37/38
20	0,76/0,80[b]	0,82/0,88	0,86/0,87	29/33[b]	39/40	36/39
30		0,89/0,91	0,98/0,89		42/43	38/41
40		0,96/0,93	1,29/0,95		43/45	41/43
50		1,16/0,98	1,47/0,97		43/46	37/45
60		1,19/1,00	1,32/0,99		43/47	38/47
70		1,10/1,05	1,21/1,07		44/47	40/48
80		0,99/0,98	1,09/1,04		43/48	42/47
90		0,97/0,99	1,11/1,09		43/47	43/48

[a] Indices greater than 1,20 represent splenomegaly. The number of tested chimeras was 4–7per point. [b] Recipients of thymus cells died within 21 days after irradiation and cell inoculation. [c] Each point represents the mean of determinations made on 5 to 9 chimeras.

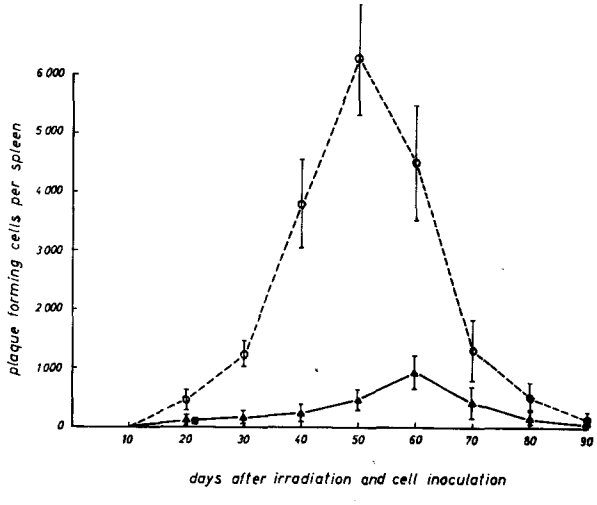
Hosts inoculated with bone marrow cells alone did not show a significant death rate which might be due to a GVH reaction within an observation period of 90 days. From the 50th to 60th day after cell inoculation, however, the spleen to body weight ratio altered from that of isologous substituted controls. The calculated spleen index was at the upper level of the normal value in most of the chimeras examined during this period (Table). The number of donor type PFC slowly increased in these chimeras from the 30th to 60th day and decreased thereafter (Figure).

Thymus and bone marrow cells combined caused in 14% a marked GVH reaction with splenomegaly, loss in body weight, dermatitis, diarrhea and anemia, which was followed by death. In 86% only a temporary GVH reaction was developed by this cell combination. Analysis of the Figure shows that in these hosts between the 30th and 50th day the number of donor type PFC increased. During this interval the spleen index of the chimeras was greater than that of the controls and the hematocrit was reduced to below the control values (Table). Further analysis of the data show that donor PFC began to decrease between the 50th to 60th day after cell inoculation. Later on the spleen size became normal and anemia was reduced but remained slightly below the control values.

Discussion. The present data demonstrate that thymus or bone marrow cells alone from newborn rats were not capable of causing a measurable graft versus host reaction. On the other hand, the combination of these two cell types produced marked GVH symptoms so that it may be concluded that this GVH reaction is the result of a synergistic action between donor thymus and bone marrow cells. It is not clear whether the mechanism involved in GVH reaction is to be understood only in terms of cellular or humoral immunity. The appearance of special cells (pyroninophilic cells) with a destructive power in the enlarged spleen and other organs[22, 23] points to a process in which cellular immunity is involved, and the basic mechanism of this process may be different from that seen in the humoral immune response[8, 9, 24, 14]. On the other hand, the increasing number of hemolysin-forming cells in temporal accordance with the increase of GVH symptoms, as has been shown in earlier experiments[20], points to a complex mechanism in which the hosts' histocompatibility antigens are also affected by humoral antibodies. It cannot be decided which mechanism (the one based on cellular, or on humoral immunity) causes the GVH reaction for the most part; but it seems possible that the structure of organs is destroyed in the way of cellular immunity, whereas rapidly growing cellular systems (erythropoietic system; hair building cells; germ cells) are mainly affected by humoral immunity.

As indicated by this study the latter point of view may be supported by the striking correlation of increasing donor PFC and increasing anemia (comparison of the table and the figure) and the decrease in the number of donor PFC and the decrease of anemia.

Moreover, it is worth noting that cells at an early stage of development, in which tolerance is relatively easy to induce[25, 26], are capable of causing a GVH reaction[27].



Rat specific PFC produced in the spleens of lethally irradiated mice injected with thymus cells (■---■), bone marrow cells (▲—▲) and a mixed inoculum of thymus and bone marrow cells (○---○) from newborn rats. The chimeras were sensitized to SRBC with 0.2 ml of a 20% SRBC suspension per animal 5 days prior to the PFC assays. The standard errors are denoted by the mean of determinations made on 4 to 7 mice.

Zusammenfassung. Thymus- und Knochenmarkzellen von neonatalen Ratten wurden in letal bestrahlte Mäuse inoculiert. Thymus- oder Knochenmarkzellen allein erzeugten keine messbare immunologische Reaktion gegen den Wirt. Eine Kombination beider Zellarten verursachte jedoch eine GVH-Reaktion (Synergismus). Neben einer Schädigung durch zelluläre Immunreaktionen scheinen besonders die Wechselgewebe (erythropoetisches System etc.) des Wirtstieres auch auf humoralem Wege angegriffen zu werden.

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19. September 1972.

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[27] Some of the experiments were carried out at the Institute for Experimental cancer Research, DKFZ, Heidelberg. This work was supported in part by the Deutsche Forschungsgemeinschaft.
[28] I am very grateful to Professor H. Friedrich-Freksa for helpful discussions. Thanks are also due to Mrs. G. Riedle for excellent technical assistance and to Dr. S. B. Pal for reading the manuscript.

Eradication of Lymphoma Cells with Allogeneic Immune Peritoneal Cells

One of the most interesting developments in the field of tumour-immunology is the evidence that both allogeneic immune peritoneal macrophages[1–3] and lmphocytes[4] are cytotoxic towards tumour cells. Den Otter et al.[3] have shown in in vitro experiments that allogeneic immune peritoneal macrophages from C57BL mice are very cytotoxic towards DBA/2 derived target cells (SL2 lymphoma cells) resulting in lysis of the target cells within 9 h. On