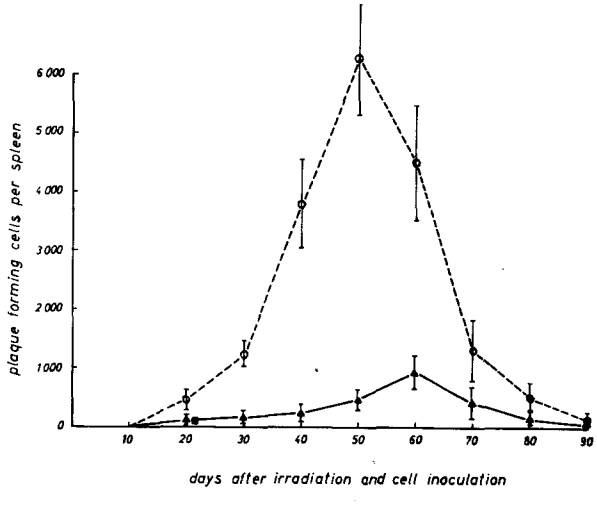
Hosts inoculated with bone marrow cells alone did not show a significant death rate which might be due to a GVH reaction within an observation period of 90 days. From the 50th to 60th day after cell inoculation, however, the spleen to body weight ratio altered from that of isologous substituted controls. 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.

[22] J. Barchilon, R. K. Gershon, Nature, Lond. *227*, 71 (1970).
[23] A. Davies and S. Doak, Nature, Lond. *187*, 610 (1960).
[24] H. R. Hilgard, J. exp. Med. *130*, 317 (1970).
[25] M. Hasek, A. Lengerova and T. Hraba, Adv. Immun. *1*, 33, (1961).
[26] G. Nossal and C. Austin, J. Immun. *95*, 665 (1965).
[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.
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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

Table I. Effect of simultaneous injection of DBA/2 SL2 lymphoma cells and C57BL allogeneic peritoneal exudate cells into DBA/2 mice

No. of SL2 cells injected[a]	No. of survivors at 35 days (actual survival time in days in parenthesis) following treatment with[b]		
	'Immune' exudate cells	'Non-immune' exudate cells	Medium only
2×10^3	5/5 (48, 52, 150, 178, 180)	0/5 (18.2 ± 0.6)	0/5 (18.6 ± 3.5)
2×10^4	3/5 (50, 63, 101)	0/5 (16.2 ± 0.3)	0/5 (15.8 ± 1.5)
2×10^5	0/5 (20.4 ± 6.7)	0/5 (14.0 ± 1.1)	0/5 (13.4 ± 1.2)
2×10^6	0/5 (13.0 ± 2.0)	0/5 (12.6 ± 1.2)	0/5 (12.8 ± 1.2)

[a] Intraperitoneal injection in 1 ml of Fischer's medium into normal DBA/2 mice. [b] Treatment consisted of i.p. injection of 2×10^6 macrophages (plus lymphocytes) from C57BL mice ('immune' = immunized 20 days earlier by i.p. injection of 10^7 SL2 cells; 'non-immune' = exudate cells from not immunized normal C57BL mice) or Fischer's medium 2 h following challenge with tumour cells.

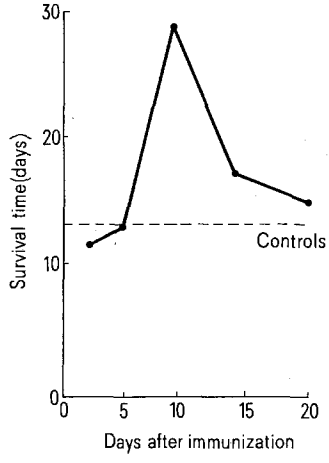
the basis of these findings we started a series of experiments to develop an optimal system for the eradication of tumour cells with allogeneic immune peritoneal cells.

Materials and methods. C57BL mice were immunized with one i.p. injection of 10^7 DBA/2 derived SL2 lymphoma cells. Immune peritoneal exudate cells were collected 20 days after immunization by washing the peritoneal cavity with 5 ml Fischer's medium[5]. Cells were centrifuged and resuspended in Fischer's medium at a concentration of 2×10^6 peritoneal macrophages (and an approximately equal number of lymphocytes)/ml. 1 ml of this suspension was injected i.p. into DBA/2 mice, 2 h after i.p. injection of $2 \times 10^3 - 2 \times 10^6$ SL2 cells in 1 ml Fischer's medium.

There were 2 groups of controls. The first group was injected i.p. with 1 ml of Fischer's medium apart from lymphoma cells and the second group was injected i.p. with 2×10^6 normal C57BL allogeneic macrophages (and an approximately equal number of lymphocytes) 2 h after i.p. injection of the SL2 cells.

To study the influence of the immunization time, 2×10^6 immune peritoneal macrophages (plus lymphocytes), collected at various times after i.p. immunization with 10^7 SL2 cells were injected i.p. into DBA/2 mice 2 h after i.p. injection of 2×10^5 SL2 cells.

In a 3rd experiment, hyperimmune C57BL peritoneal exudate cells were used. C57BL mice were immunized at day 0, 10, 17, and 22 with 10^7 SL2 i.p. Hyperimmune exudate cells were collected at day 24. Cells were centrifuged and resuspended in Fischer's medium. 1 ml of this suspension containing 2×10^6 peritoneal macrophages (and an approximately equal number of lymphocytes) was injected i.p. into DBA/2 mice 2 h after injection of $2 \times 10^3 - 2 \times 10^7$ SL2 lymphoma cells.



Influence of immunization time on immunity of peritoneal exudate cells. Peritoneal immune C57BL cells were harvested 2, 5, 9, 14 or 20 days after immunization with 10^7 SL2 i.p. 2×10^6. Immune macrophages (and an approximately equal number of lymphocytes) were injected i.p.into DBA/2 mice 2 h after these mice were injected i.p. with 2×10^5 SL2. Immunity is measured in terms of the number of days of survival. Each result is the mean of 5 mice.

[1] G. A. GRANGER and R. S. WEISER, Science *145*, 1427 (1964).
[2] G. A. GRANGER and R. S. WEISER, Science *151*, 97 (1966).
[3] W. DEN OTTER, R. EVANS and P. ALEXANDER, Transplantation *14*, 220 (1972).
[4] B. BENNETT, J. Immun. *95*, 656 (1965).
[5] G. A. FISCHER, Ann. N. Y. Acad. Sci. *67*, 673 (1958).

Table II. Effect of simultaneous injection of DBA/2 SL2 lymphoma cells and allogeneic C57BL hyperimmune peritoneal exudate cells into normal DBA/2 mice

No. of SL2 cells injected[a]	No. of survivors at 35 days (actual survival time in parenthesis) following treatment with[b]		
	'Hyperimmune' exudate cells	'Non-immune' exudate cells	Medium only
2×10^3	4/5 (37, 69, 97, one is still alive)	0/5 (14.9 ± 1.1)	0/5 (14.0 ± 0.9)
2×10^4	3/5 (38, 50, one is still alive)	0/5 (13.6 ± 0.8)	0/5 (13.0 ± 1.0)
2×10^5	4/5 (37, 38, 42, 62)	0/5 (12.4 ± 1.2)	0/5 (12.8 ± 0.8)
2×10^6	2/5 (37, 56)	0/5 (11.0 ± 0.4)	0/5 (10.6 ± 0.8)
2×10^7	0/5 (13.2 ± 4.0)	0/5 (10.5 ± 0.2)	0/5 (10.3 ± 0.4)

[a] Intraperitoneal injection in 1 ml of Fischer's medium into normal DBA/2 mice. [b] Treatment consisted of i.p. injection of 2×10^6 macrophages (plus lymphocytes) from C57BL mice ('hyper-immune' = immunized by i.p. injection of 10^7 SL2 cells at day 0, 10, 17, 22. Cells were collected at day 24; 'non-immune' = exudate cells from not immunized normal C57BL mice) or Fischer's medium 2 h following challenge with tumour cells.

Results. 20 days immune peritoneal cells. The results of the first experiment show that significant differences in survival time can be obtained with 1 i.p. injection of peritoneal exudate cells containing 2×10^6 allogeneic immune macrophages, collected 20 days after i.p. immunization with 10^7 SL2 (Table I). 3 mice injected with 2×10^4 SL2 cells, and all mice injected with 2×10^3 SL2 cells survived for more than 35 days. Survival times of these mice challenged with 2×10^4 and 2×10^3 SL2 tumour cells varied from 50–101 and 48–148 days, respectively. The survival times of the controls injected with 2×10^3 – 2×10^6 SL2 cells, and 'treated' with 1 ml Fischer's medium apart from lymphoma cells varied from 18.6 ± 3.5 – 12.8 ± 1.2 days, respectively. These figures were not significantly different from those of controls injected with 2×10^6 non-immune allogeneic C57BL macrophages (plus lymphocytes) after challenge with 2×10^3 – 2×10^6 SL2 lymphoma cells.

Influence of immunization time. The influence of the time between immunization and collection of the exudate cells is shown in the Figure. Optimal immunity is found at about 9 days after immunization. Controls injected i.p. with 1 ml of Fischer's medium apart from 2×10^5 SL2 lymphoma cells died after 13.4 ± 0.6 days. The fact that mice treated with peritoneal cells collected 2 and 5 days after immunization died earlier than the controls can be explained by the fact that SL2 cells were not completely rejected at the time of collection of the exudate cells.

Hyperimmunity. Hyperimmune peritoneal cells can eradicate more lymphoma cells than 20 days immune peritoneal cells (Table II). 2 animals even survived a dose of 2×10^6, and 4 mice a dose of 2×10^5 SL2 lymphoma cells for more than 35 days. Survival times from the mice challenged with 2×10^6 and 2×10^5 lymphoma cells varied from 37–56 and from 37–62 days respectively.

We would have expected that 5 out of 5 mice survived an injection with 2×10^3 or 2×10^4 SL2 cells after treatment with hyperimmune peritoneal cells, instead of 4 and 3 out of 5. This result is possibly due to the presence of SL2 cells in the peritoneal cavity of C57BL mice at the time of collection of the hyperimmune cells. Survival times of the mice challenged with 2×10^4 or 2×10^3 SL2 cells varied from 37–97 and 38–50 days, respectively. From both groups 1 mouse is still alive (220 days).

Discussion. Peritoneal macrophages and lymphocytes were not separated in these experiments. It would not be surprising to learn from further experiments that the killing effect is the result of a synergistic effect of macrophages and lymphocytes. However, much more important than the question which cell type is responsible for the effect, is the fact that considerable differences in survival time did occur with immune or hyperimmune exudate cells.

The important of these results is clear if the number of 2×10^6 SL2 cells that can be eradicated is compared with the maximum number of cells that can be obtained from a transplantation mouse being 5×10^8 SL2 cells. Furthermore, it has always been stressed that with immunotherapy only a very small number of tumour cells, no more than 10^3–10^5 cells, can be eradicated[6–8].

Further experiments about optimal conditions for eradication of lymphoma cells with allogeneic immune and hyperimmune peritoneal cells, e.g. with hyperimmune peritoneal exudate cells collected 9 days after the last shot, are in progress.

Zusammenfassung. DBA/2 Mäuse wurden nach Injektionen mit SL2 Zellen mit allogenen, immunen und hyperimmunen Exsudat-Zellen aus dem Peritonealraum behandelt. Diese Behandlung ergab eine Eliminierung von 2×10^6 SL2 Zellen.

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[6] G. Mathé, Br. med. J. *4*, 487 (1970).
[7] P. Alexander, Br. med. J. *4*, 484 (1970).
[8] P. Alexander, D. I. Connell and Z. B. Mikulska, Cancer Res. *26*, 1508 (1966).

A New Lymphocyte Transforming Factor Derived from the Lysosomes of Polymorphonuclear Leucocytes

The lysosomes of polymorphonuclear (PMN) leucocytes have been implicated as mediators of tissue injury and inflammation[1], their content release being triggered by the formation of antigen-antibody complexes and by phagocytosis[2–4]. Some correlation has been shown between a decrease in lysosome enzyme activity of PMN leucocytes and the number of blastic cells in the lymph nodes draining the area of allogeneic skin graft in rabbits[5]. In this report data will be presented which suggest an important role of the lysosomes of PMN leucocytes in the processes of the lymphocytes transformation in vitro.

Materials and methods. The investigations were carried out on lymphocytes and lysosomes isolated from PMN leucocytes, macrophages, lymphocytes and liver cells. The induction with 0.1% glycogen solution of PMN-rich peritoneal exudate, the collection of the cells and separation of lysosomes was performed according to described methods[6,7]. PMN leucocytes were obtained from the peritoneal cavity of guinea-pigs and rabbits according to Cohn[6]. Human peripheral blood leucocytes were obtained after red blood cells sedimentation with high molecular dextran. Macrophages were obtained from the peritoneal cavity of guinea-pigs[7]. The final preparation contained over 87% macrophages. Lymphocytes were obtained from spleen and lymph nodes of guinea-pigs and rabbits, human lymphocytes were obtained from peripheral blood. Separation of lymphocytes (99% pure) was performed according to Rabinowitz technique[8]. Lymphocytes lysosomes were isolated according to Hirschhorn et al.[9].

[1] C. G. Cochrane, *Advances in Immunology* (Academic Press, New York 1968), vol. 9, p. 97.
[2] C. G. Cochrane, W. O. Weigle and F. J. Dixon, J. exp. Med. *122*, 99 (1959).
[3] H. Z. Movat, D. R. L. Macmorine and Y. Takeuchi, Int. Arch. Allergy *40*, 218 (1971).
[4] D. Hawkins and B. Sc. Peters, Lab. Invest. *24*, 483 (1971).
[5] H. Tchórzewski, Z. Domański and B. Tazbir-Przybylska, Ann. Immun., *4*, 45 (1972).
[6] Z. A. Cohn and J. G. Hirsch, J. exp. Med. *112*, 983 (1960).
[7] Z. A. Cohn and E. Wiener, J. exp. Med. *118*, 1009 (1963).
[8] Y. Rabinowitz, Blood *23*, 811 (1964).
[9] R. Hirschhorn, G. Britinger, K. Hirschhorn and G. Weissmann, J. Cell Biol. *37*, 412 (1968).