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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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].

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Table 1. Transformation rate of guinea-pig lymphocytes in crude lysosomal material (CLM) stimulated cultures

Blast percentages against 500 examined cells			Crude lysosomal material dosis				
Crude lysosomal material	Control	PHA	0.2 µg/ml	5.0 µg/ml	10.0 µg/ml	20.0 ug/ml	100.0 µg/ml
PMN leukocytes	5.6 ± 2.0 (2)	43.6 ± 5.1 (8)	14.3 ± 1.0 (4)	33.1 ± 8.2 (7)	28.8 ± 3.0 (10)	27.2 ± 10.5 1(6)	15.0 ± 5.0 (20)
Lymphocytes	4.2 ± 1.0 (4)	39.4 ± 7.5 (8.5)	6.1 ± 3.1 (9)	7.8 ± 2.3 (11)	3.8 ± 1.4 (11)	8.2 ± 4.0 (18)	6.0 ± 3.5 (18)
Macrophages	6.3 ± 2.1 (3)	47.5 ± 7.2 (7)	11.0 ± 2.0 (8)	18.2 ± 8.4 (14)	13.7 ± 8.5 (16)	17.1 ± 5.0 (21)	15.2 ± 4.0 (20)
Liver cells	7.3 ± 4.0 (3)	47.5 ± 7.2 (7)	15.4 ± 4.8 (12)	16.1 ± 5.2 (14)	12.6 ± 7.0 (14)	13.0 ± 4.7 (18)	12.4 ± 4.5 (23)
Supernatant of lysomal granules of							
PMN (cell plasma fraction	5.6 ± 2.0	43.6 ± 5.1	—	6.2 ± 2.2 (18)	11.0 ± 3.0 (18)	10.0 ± 4.5 (25)	7.2 ± 3.1 (30)

Percentages of cells trypan blue permeable in parenthesis.

The lysosomal granules were washed with PBS twice prior to being depleted of their contents by freezing and thawing, repeated 4 times. Protein content in all investigated fractions was measured by the LOWRY et al.[10] method. Liver cells lysosomes were isolated according to WEISSMANN et al.[11]. The specificity of crude lysosomal material (CLM) was determined by the assay of acid phosphatase[12] and acid protease[13].

Crude lysosomes material was applied to DEAE cellulose column equilibrated with 0.01 *M* phosphate buffer pH 7.8[14]. The fractions were tested for their enzymatic and lymphocyte transformation activity. Esterolytic activity was measured on *p*-tosyl-arginine methyl ester (TAMe)[15]. The lymphocytes transforming activity was measured on the culture of lymphocytes[16,17].

Blasts were counted under the light microscope according to the criteria of CHESSIN et al.[16]. The results were expressed in terms of the blast percentages of 500 examined cells. Viability of cells within the cultured suspensions was determined by trypan blue exclusion[18]. The column eluated fractions were tested for lymphocyte transformation activity in concentration of 5 µg/ml. Controls represent lymphocytes incubated in culture medium. Phytohemagglutinin (PHA) Difco 10 µg/ml was used in experiments.

The active lymphocyte transforming material eluated from DEAE cellulose column was separated by Sephadex

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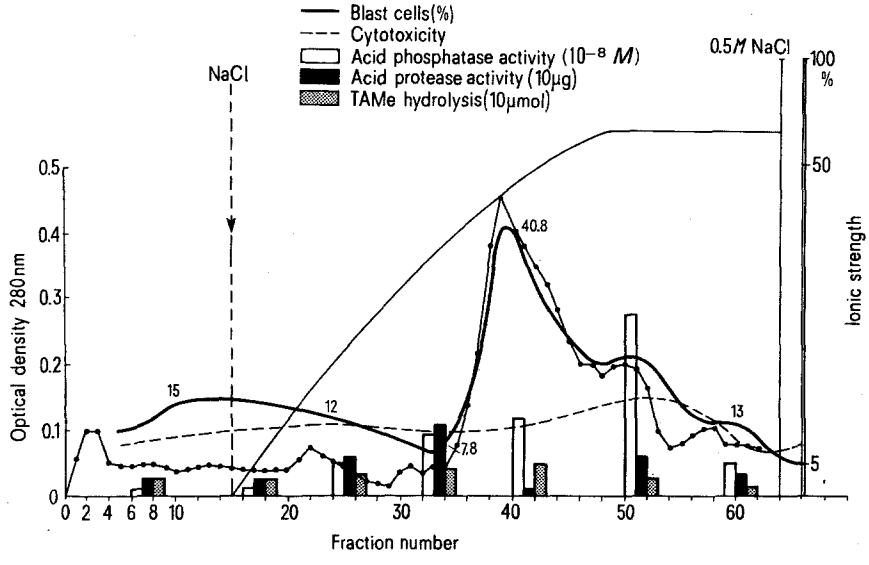

DEAE cellulose chromatography of guinea-pig PMN leucocytes crude lysosomes material (CLM) 2×25 cm column. The eluated fractions were dialyzed against 0.85% NaCl, condensed by evaporation in + 4 °C and tested to enzymatic and transformation activity.

G-100 gel chromatography into 2 fractions: a low and high molecular one[19].

Results. The lysosomal hydrolases of CLM showed an average increase in specific activities over the cell homogenate 4–5 fold. The results of guinea-pig lymphocyte transformation in PHA, CLM and PMN leucocytes plasma material stimulated cultures are summarized in the Table. Lysosomal material separated from macrophages, lymphocytes and liver cells do not shown similar transformation activity in vitro. The heterogeneic crude lysosomal material of PMN leucocytes does not stimulate lymphocytes cultures in vitro (unpublished). The lymphocyte suspension was adjusted to a concentration $2–4 \times 10^6$ cells/ml in all experiments (Figure).

Fractions eluated from DEAE cellulose column were dialyzed. Material from these fractions was added to the lymphocyte cultures in quantities of 5 µg/ml. Studies with DEAE cellulose eluated fractions indicated that stimulatory effect on lymphocyte cultures was shown by PMN leucocytes lysosomal material. This suggests that only the PMN leucocytes possess the lymphocyte transforming activity.

Discussion. The factor described above stimulating lymphocyte transformation in vitro, can also perform some important functions in vivo. The response of cells of the lymphoid system in the course of phagocytosis may be unspecifically intensified after the release of stimulators known to be present in lysosomes of the PMN leucocytes. This assumption is supported by investigations on the role and mechanism of the adjuvants in immunological processes. Some adjuvants are labilizators of lysosomal granule membranes and activiting their contents[20]. The results obtained with fraction eluated from DEAE cellulose column indicate that lymphocyte transformation factor does not possess high activity level of the proteases which play an important role in blastic transformation of lymphocytes[17,21]. Activity of lymphocyte transforming factor described above cannot therefore be of an enzymatic nature.

The findings reported above indicate that the extracellular release of lysosomal constituents may contribute, not only as is generally believed, to the induction of vascular phenomena of inflammation but also to the lymphocyte transformation.

Résumé. On décrit le facteur protéique stimulant in vitro la transformation blastique des lymphocytes allogènes. La présence de ce facteur ne put être établie à l'intérieur des lysosomes des macrophages, des lymphocytes, des cellules hépatiques, ni dans les fractions des granulocytes polymorphonucléaires contenant le plasma cellulaire dépourvu d'autres structures à la suite d'une centrifugation. On discute le rôle présumé du facteur étudié in vivo.

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Disappearance of Radioactivity from Plasma Following Intravenous Administration of Tritiated Thyrotrophin-Releasing Hormone (Ro 8-6270) to Man

Thyrotrophin-releasing hormone (TRH) is a simple tripeptide, pyroglutamyl-histidyl-proline-amide, secreted by the hypothalamus which stimulates the release of thyrotrophin (TSH) from the anterior pituitary into the peripheral circulation. Since the recent isolation and synthesis of TRH[1–5] considerable interest has been directed towards the importance of the hormone both clinically and in the research laboratory.

Studies on the distribution and excretion of radioactivity following administration of labelled TRH to rats and mice have been reported[6,7]. The results of these studies have shown that the highest accumulation of radioactivity occurred in the pituitary, liver and kidneys. Assays of urinary radioactivity has indicated that up to 40% of the radioactive dose was excreted in the urine within 1 h. Redding and Schally[8] have recently shown that, following i.v. injection of TRH labelled with ^{14}C-histidine to rats, the plasma ^{14}C-activity had a disappearance half-life of about 4 min up to 6 min following administration.

We wish to report here on studies carried out to determine the disappearance rate of radioactivity in plasma and its excretion in urine following intravenous administration of tritium labelled TRH to man.

Materials. Pyroglutamyl-2,5-^{3}H-histidyl-proline-amide hydrochloride with a specific activity of 9.15 mC/µ*M* was supplied by F. Hoffmann-La Roche and Co. AG., Basel, Switzerland. The preparation and purification of the radioactive tracer will be reported elsewhere. The radioactive doses for i.v. injection were prepared by adding 120 µC of the tracer in 0.1 ml of ethanol to vials containing 200 µg of unlabelled TRH (Ro 8-6270) as a solution in 2 ml of isotonic saline.

Methods. Two normal males and two normal females each received 200 µg (120 µC) of tritium labelled TRH by rapid i.v. injection. Blood samples were collected in heparinized tubes at the times indicated in the Figure and the plasma separated by centrifugation and stored at –20° until analysis. Urine was collected daily for two days.

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