the patients have had about a pound of flesh removed. This, of course, tended to be slightly less in the *Thompson* operation than in the *Homans'* because the buried flap retained weight 6, 7 or maybe 8 ozs.

There was only a slight relation between necrosis and the weight excised. Patients with large excisions regularly had some degree of flap necrosis but this was balanced by a number of cases with necrosis after excision of small weights. Possibly those with minor degrees of oedema had flaps thinned to a precarious degree in order to obtain reduction.

Assessment of Results

Lastly we attempted to assess the results, although it was inevitably a somewhat arbitrary matter. We have classified the patients into poor, moderate and good. In the "poor" (13 operations) the leg was the same size as before operation and the surgeon doubted its worth. But on the whole almost all the patients were glad that they had the operation done. They appeared to be less critical than the surgeons. There were very few indeed who said that they thought it was not worthwhile.

This is of course a very arbitrary classification.

Forty five patients were put in the "moderate" class where the major circumference six months or more after operation was less than pre-operative, the patients was pleased and wished for further operation on the contralateral limb or elsewhere on the same limb. In the last group classified as "good" there was unequivocal satisfaction and delight all round. These of course were chiefly patients with very large limbs.

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Transplantation Models Using the Regional Lymph Node

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Summary

Several transplantation models, using the regional lymph node, to study the transplantation reaction in strictly defined and simple conditions were devised. Lymphoid cells were transplanted to inbred rats and mice at the sites drained by one regional lymph node; the experimental design was chosen so as to permit theoretically a one-way reaction, either the host-versus-graft (HVG) or the graft-versus-host (GVH) reaction.

The changes in the lymph nodes draining the site of cell injection (weight increase, histology, lymphocyte activation) were very similar in both transplantation reactions. They were ascribed to a humoral mediator released upon the contact of lymphoid cells from two genetically different individuals. The direct demonstration of a mediator which is capable of activating the regional lymph node in vivo introduces some new aspects into the process of sensitization after transplantation.

Studies of transplantation immunity using the classical skin grafting paid little or no attention to the possibility of a two-way reaction and to the role of the lymphatic system (2, 23). There are complicated conditions also in the theoretically one-way graft-versus-host (GVH) reactions using the transfers of lymphoid cells i.p. or i.v. to neonatal or whole-body irradiated adult recipients which are evaluated on the basis of growth retardation or the number of the animals injected and lost (3, 22) or on the basis of spleen enlargement (24). The GVH reaction of the cells injected below the renal capsule (5) or the GVH reaction in vitro (1) has some specific features and technical problems too.

In our experimental models a defined amount of isolated lymphoid cells was transplanted subcutaneously to the hind footpads of inbred mice and rats, i.e. to the region drained by one regional lymph node. These models permit theoretically a one-way GVH or HVG reaction and allow the study of several aspects of the transplantation reaction. The finding of a humoral factor released during a short-term contact of the allogeneic lymphoid cells and activating in vivo the regional lymph node introduces new aspects into the process of sensitization after transplantation, although its role in the development of specific effector mechanisms of transplantation immunity remains obscure.

Materials and Methods

Rats of the LEWIS and AVN inbred strains, differing at the strong Rt H-1 locus as well as at multiple non H-1 loci, and their F_1 hybrids ($LWAF_1$), and mice of the congenic inbred strains C57BL/10Sn (B10), B10.D2, B10.LP, 40NX and their F_1 hybrids were used in almost all experiments. The animals, both males and females, were 2 to 7 months old. Lymph node cells (8, 10, 13, 16, 17) or blood (0.3 ml) obtained by cardiac puncture (11, 12) were injected s.c. into the hind footpads. Seven days later, the right and left popliteal lymph nodes were carefully excised, and the enlargement index (E.I.), i.e., the ratio of the weight of the right node to that of the left, uninjected side was calculated. For histological examination, the lymph node cells were fixed in SUSA solution according to *Heidenhain* and embedded in paraffin. Serial sections 4 μ thick were stained with haematoxylin-eosin.

To determine lymphocyte activation, the lymph node cells were released into drops of normal non-inactivated isologous serum. Dry smears were treated for 10 min. with toluidine blue, pH 5 (25), staining ribonucleoprotein structures. The lymphocytes with compact nucléoli or nucleoli with nucleolonemas indicating the synthesis of ribonucleic acid (RNA) were considered active (15).

For the demonstration of the lymph node activating mediator, the lymph node cells from mice of two genetically different mouse strains were carefully isolated and cultivated in culture medium 199 (Institute of Sera and Vaccines, Prague): 10×10^6 cells from each cell partner in the mixed allogeneic cell cultures, or 20×10^6 cells from one cell partner in the control syngeneic cell cultures, under the conditions of 4×10^6 cells/ml at 37°C. Four or 8 hr later, culture supernatants were harvested, lyophilized, and resuspended in 1/10 the original volume. The supernatants (0.1 ml) from allogeneic and syngeneic cell cultures were injected s.c. into the right hind footpads of B10 mice or F_1 hybrids of the strains whose cells were cocultivated (Table 1).

The special techniques of skin grafting, irradiation, etc. were described in the papers cited. The significance of the differences between the values obtained was determined by the t-test or nonparametric range Mann-Whitney U-test in the Computer Centre of the Institute for Clinical and Experimental Medicine, Prague.

Results

Model 1. The regional graft-versus-host reaction for testing the immunological reactivity of injected cells. — Lymph node cell suspensions from LEWIS rats were injected s.c. into the right hind

Table 1 Enlargement indices (E.I. ± s.d.) of the popliteal lymph node 7 days after syngeneic transfer, and in the HVG or GVH situations in 187 recipient rats (with permission of Folia biol. [Praha]) (8).

No. of cells injected x 10^6	LW – LW control	HVG	GVH
1.8 – 7	1.8 ± 2.1	2.8 ± 1.6	4.8 ± 3.9
10 – 25	2.6 ± 2.5	5.5 ± 2.8	15.9 ± 4.6
35 – 80	2.7 ± 2.2	5.3 ± 2.2	

Heavily irradiated donor cells induced no reaction.

footpads of $LWAF_1$ rats. Seven days later, the weight of the draining popliteal lymph nodes increased more than fifteenfold (Table 1). The increase correlated with the number of the injected cells and their immunological reactivity. Heavily irradiated cells (2.000 R before administration) and syngeneic cells from antigenically identical donors and recipients failed to elicit such a reaction. The weight of the contralateral lymph nodes remained unchanged in all cases. The details were published elsewhere (8).

Model 2. Capability of the blood to react against transplantation antigens

The preceding test was modified for testing the capability of non-isolated lymphocytes in whole blood to elicit the GVH reaction (11). Erythrocytes were found not to distort the reaction (E.I. = 1.6 ± 0.4 (s.e.) in 8 syngeneic recipients). In $LWAF_1$ hybrids, injected with 0.3 and 0.15 ml of heparinized blood from LEWIS rats, the draining popliteal lymph nodes of 28 recipients increased, the E.I. being 9.1 ± 1.0 (s.e.) and 4.5 ± 1.0 (s.e.), respectively. One injection of antilymphocyte serum into the LEWIS rat donor reduced the enlargement indices in $LWAF_1$ hybrids to the values observed in the syngeneic recipients. On the other hand, in the donors sensitized against the recipients by a skin graft, the capability of the blood to react against the donor's transplantation antigens increased 7 days after the transplantation (11).

Model 3. Transplantation host-versus-graft reaction (HVG) in the regional lymph node

The changes in the popliteal lymph node weight were investigated also in the opposite genetical situation, i.e., in LEWIS recipients injected with F_1 hybrid cells, which theoretically permits a one-way HVG reaction (10). On day 7 after the injection the lymph node enlargement index was invariably significantly higher. However, the increase was never as marked as in the GVH situation (Table 1).

Model 4. Inbred strain homogeneity test

The regional lymph node weight increase after the injection of cells in the GVH or HVG situations was used to test the homogeneity of the inbred strains (9). It appears to be much simpler than the currently used test of skin graft survival. Lymph node cells from the animal of the strain tested were injected s.c. into the right hind footpads of a number of animals of the same strain (20 million cells per animal). Seven days later, the popliteal lymph nodes both on the injected and uninjected sides were isolated and the enlargement indices were assessed.

Table 2 Strain homogeneity testing

	No. of animals injected	Enlargement index ± s.d.
LEWIS – LEWIS	19	1.3 ± 0.5
AVN – AVN	18	1.8 ± 1.0
WISTAR – WISTAR (semiinbred)	12	6.7 ± 4.9
white outbred – – white outbred	18	9.9 ± 8.3

Table 2 shows that after the transfer of cells the draining nodes in the inbred strain increased only once to twice, in the semi-inbred strain 6-7 times and in the outbred animals almost 10 times. Standard deviation (s.d.) values were seen to increase simultaneously. This may be accounted for by the different genetic relationship of the donor-recipient pairs.

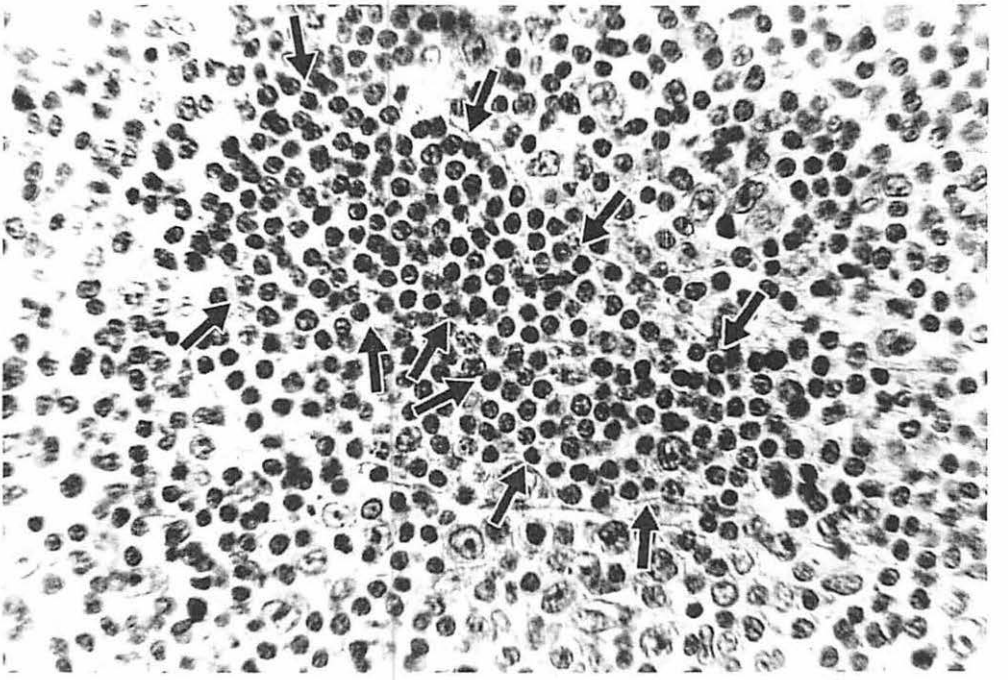

Fig. 1 Rat popliteal lymph node, 7th day of GVH reaction induced by the injection of LEWIS lymphocytes into a F_1 hybrid: the intermediary sinus distended (arrows) by a lymphocytic plug. Haematoxylin-eosin, x 120.

Model 5. Histology and lymphocyte activation in the regional lymph node after allogeneic cell transfers

The histological picture of the draining popliteal lymph nodes (16) both in the GVH and HVG reactions is characterized by accumulation of lymphocytes in the cortex, paracortex and the sinuses. In addition, the activation and proliferation of the nodal reticulum and damage of the endothelium of postcapillary venules were observed. This may affect the control of lymphocyte penetration from the venule lumina and the intensity of trapping (Fig. 1).

One of the early manifestations in the activation of lymphocytes is an increased synthesis of RNA. The high percentage of lymphocytes with compact nucleoli and nucleoli with nucleolonemas indicates the activation of RNA synthesis. On the other hand, ring-shaped nucleoli or micronucleoli are present in cells with no demonstrable RNA synthesis (25). Using this test, we have found a correlation between the time of blood lymphocyte activation and skin graft survival (12, 15). A significantly higher percentage of active lymphocytes was seen also in the popliteal lymph nodes of mice and rats 2 days after the injection of semiallogeneic cells (10×10^6) into the hind footpads.

It should be noted that in mice differing at the strong H-2 antigenic system an almost identical reaction takes place not only in the "one-way" host-versus-graft, but also in the graft-versus-host situation (17) (Fig. 2, 3). After the transfer of syngeneic cells no activation occurred (Table 3). Hence it follows that the activation of the lymph nodes detectable in the HVG and GVH reactions may be a secondary event resulting from the recognition of foreign transplantation antigens by lymphoid cells irrespective of whether the cells derived from the recipient react against the donor, or the donor cells react against the recipient. This hypothesis was confirmed by subsequent experiments.

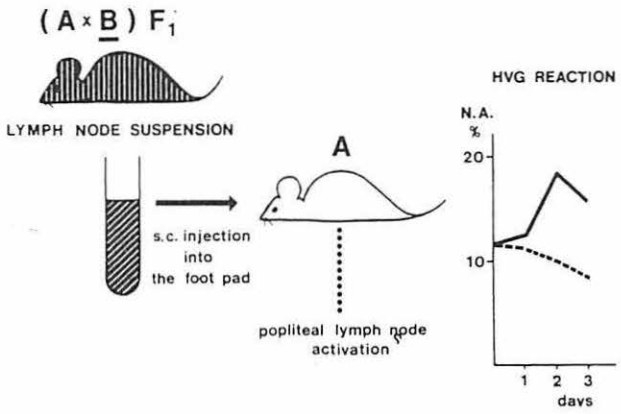

Fig. 2 Diagramatical representation of the regional HVG reaction showing the increase in the percentage of activated lymphocytes (N.A.) in the draining (——) and contralateral (----) popliteal lymph node after injection of cells.

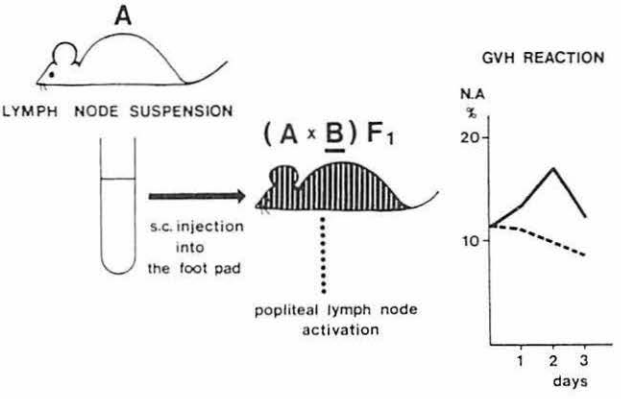

Fig. 3 Diagramatical representation of the regional GVH reaction showing an activation of popliteal lymph node comparable with the HVG situation. N.A.% = percentage of active lymphocytes in the draining (——) and contralateral (----) popliteal lymph node.

Table 3 Activation of lymphocytes in the draining and contralateral lymph nodes in the HVG and GVH reactions

Reaction Donor cells	Recipients	No. of recipients	Mean percentage of active lymphocytes DLN[a]	 CLN[b]
HVG (B10 x B10.D2)F_1	B10	5	18.4 (17-21)[c,d]	10.0 (8-15)
GVH B10	(B10 x B10.D2)F_1	5	17.5 (10-20)[d]	7.1 (2-12)
Controls B10	B10	5	9.2 (3-15)	11.8 (6-16)

a = draining lymph node
b = contralateral lymph node
c = minimal and maximal values in parentheses
d = significant difference ($p < 0.001$) with CLN and DLN of controls (Mann-Whitney U-test).

Model 6. Evaluation of histoincompatibility according to the capacity of culture supernatants to activate the regional lymph node (18).

The supernatants from 4-hr culture of lymphocytes from B10 and B10-D2 mice increased the percentage of lymphocytes with active nucleoli in the draining lymph nodes of B10 mice or (B10 x B10.D2)F_1 hybrids 24 hr after s.c. injection into the footpads. The supernatants from the 4- or 8-hr syngeneic cell cultures elicited no activation in the recipients' draining lymph nodes (Table 4).

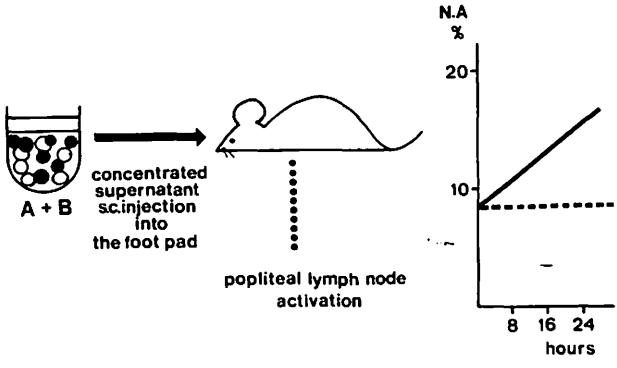

Fig. 4 Diagramatical representation of the experiment showing the existence of a soluble lymph node activating factor released upon the contact of allogeneic (A and B) lymphoid cells.

Table 4 Demonstration of lymph node activating mediator

Cultured cells	Recipients of supernatants	No. of recipients	Mean percentage of active lymphocytes DLN[a]	CLN[b]
B10 + B10.D2	B10	9	16.8 (13-25)[c,d]	8.0 (7-12)
B10 + B10.D2	(B10 x B10.D2)F_1	7	17.0 (10-21)[d]	7.1 (4- 9)
B10	B10	6	7.6 (5-10)	8.1 (7-10)
B10	B10.D2	6	8.5 (6-13)	8.5 (5-13)

a = draining lymph node
b = contralateral lymph node
c = minimal and maximal values in parentheses
d = significant difference with CLN ($p < 0.001$) and with DLN of animals treated with supernatants from syngeneic cell cultures ($p < 0.01$ or 0.05 – Mann-Whitney U-test).

The activating effect of the supernatants from mixed cultures of allogeneic lymphocytes may not be attributable to the release of antigenic material to the medium, because the response of the recipient was found not only in mice of one of the original donor strains, but also in the F_1 hybrids of both strains having no antigenic deficit in comparison with the cultivated cells. A mediator was released upon the contact of and transplantation antigen recognition by allogeneic cells, which may influence additional lymphocytes and/or their circulation.

Discussion

Using simplified and strictly defined models based on changes in the regional lymph node, we studied mechanisms of transplantation immunity. We showed that, both in the GVH and HVG genetic combinations, the administration of allogeneic cells into the hind footpads results in a significant enlargement of the draining lymph node and its significant activation. Enlargement observed in the GVH reaction, reported independently also by *Ford* and co-workers (7), has been accepted as a routine procedure for testing the immunological reactivity of injected cells against recipients' histocompatibility antigens (12, 13, 26). Moreover, the enlargement may serve as a test of the homogeneity of inbred strains. The procedure is much simpler than the hitherto used skin grafting. The GVH reaction induced by the lymphocytes of the whole blood may be useful in the studies of the kinetics of sensitization or of the effect of immunosuppressive drugs (11, 12).

Activation of the regional lymph nodes in transplantation is assumed to be triggered either by the donor transplantation antigens which are released from the graft and enter the regional lymph nodes through the lymphatics (4, 20), or by the recipient cells sensitized by the donor transplantation antigens during their passage through the graft and subsequently differentiated

in the draining lymph node (21, rev. 19). Our findings of lymph node activation in the HVG and GVH transplantation reactions (8, 10, 16, 17) indicate a potentially activating effect of a soluble substance released during the contact of allogeneic cells. Thereupon the substance rapidly spreads, apparently via the lymphatics and as shown by the histological picture of the affected node, probably traps the circulating recipient cells into the regional lymph node (6, 16) and activates all its components, including the reticulum (16). Following a 4-hr cocultivation of allogeneic cells in vitro we obtained supernatants which activated the lymph node in vivo similarly as do the incompatible cells (17, 18). The mediator may act as one of the aplification mechanisms which ensure a quick onset of transplantation immunity. It may explain the involvement of non-sensitized cells in the infiltration and destruction of allografts (14). The role of the mediator in the specific effector mechanism of transplantation immunity remains obscure and is under study. We believe that the simplified transplantation models using the regional lymph node may help in clarifying the mechanisms involved in the transplantation reactions.

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International Society of Lymphology

The VI. International Congress of Lymphology will be held in PRAHA/CSSR in summer 1977. This decision has been made at the General Assembly meeting of the International Society of Lymphology in RIO DE JANEIRO.

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LETTER FROM THE EDITOR:

A survey of publications dealing with almost any aspect of science leads one to recall Santayana's timeless comment regarding scientific inquiry in general: "a patient siege laid to the truth . . . as by an army of ants". With respect to those who study the lymphatic system, the "army" is small and the loss of even one contributor affects the common purpose. Thus the recent deaths in Berlin of Drs. K. Muller and J. Meyer-Burg represent a particularly significant loss from our ranks.

The two men had shared an active interest in visualization and cannulation of visceral lymphatics via peritoneoscopy. Their contributions in this regard are milestones and give promise of completely new approaches to investigation in man. They will be missed and in particular by those who had the opportunity to know them personally.

Allen E. Dumont, M.D.

Diesem Heft liegt ein Prospekt des Gustav Fischer Verlages, Stuttgart, bei.
