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# The Role of Humoral Factors in the Mechanisms of Graft-versus-host Induced Host Cell Proliferation and Lymph Node Hyperplasia in the Rat

**Bent Rolstad, M.D.**

Institute of Physiology and Anatomical Institute, University of Oslo

**Summary**

The effect of humoral factors released during systemic graft-versus-host (GVH) reactions on cell proliferation and lymph node enlargement was investigated as follows:

1) Thoracic duct lymphocytes of host origin were cultured in diffusion chambers in the peritoneal cavity of rats undergoing a systemic GVH reaction. On day 7 of the response chamber cell proliferation (as judged by $^3H$ thymidine incorporation) was increased two to four fold over that seen in normal hosts. 2) Serum from (H0xDA) $F_1$ hybrid rats being repeatedly injected with parental (HO) cells contained a factor that, when preincubated with such parental cells in vitro, was able to augment their ability to produce lymph node enlargement in a local GVH reaction. These results suggest that the host cell proliferation and lymphoid hyperplasia observed during GVH reactions might be the results of long range humoral mechanisms acting alone or in addition to the direct interaction between donor and host cells.

## *Introduction*

A still unresolved problem in transplantation immunology is why the graft-versus-host (GVH) reaction, although being thought to be mainly immunologically unidirectional, often induces a lot of host cell proliferation in the lymphoid tissues (4, 9, 12, 18). This host cell activation could either be a nonspecific response to mitogenic signals froms the activated donor cells, or, alternatively, an expression of a host homeostatic mechanism trying to limit the immunological activity of the donor cells, both hypotheses having some experimental support (2, 11, 14, 15, 16, 17, 20, 21).

In either case it would be important to know whether this cell proliferation only occurred during direct contact between donor and host cells, or whether the response could be elicited by long range soluble factors released from the interacting cells. Although the existence of blastogenic factors is well documented in in vitro experiments (11, 13, 14, 21), very few experimental systems exist to test their role in vivo (15). This report gives preliminary evidence of long range humoral factors arising during systemic GVH reactions, having the capacity of both stimulating cell proliferation and augmenting lymphoid hyperplasia in vivo.

## *Material and Methods*

1. *Animals.* Adult rats of either of the inbred strains (HO(Ag-B5) and DA(Ag-B4) as well as the $F_1$ hybrid crosses between them were used.

2. *Operative procedures.* Ether anaesthesia was used in all experiments. Thoracic duct cannulation, lymph collection and lymphocyte handling was carried out as described by *Ford* (7). The popliteal lymph node assay of *Ford* (8) was used to determine GVH activity.

3. *Diffusion chamber technique.* A routine technique in this laboratory (1) was adopted for the present purpose as follows: 2–4 chambers (Millipore filters, pore size 0.22 $\mu$m) each containing $0.5 \times 10^6$ (HO x DA)$F_1$ thoracic duct lymphocytes (TDL) suspended in

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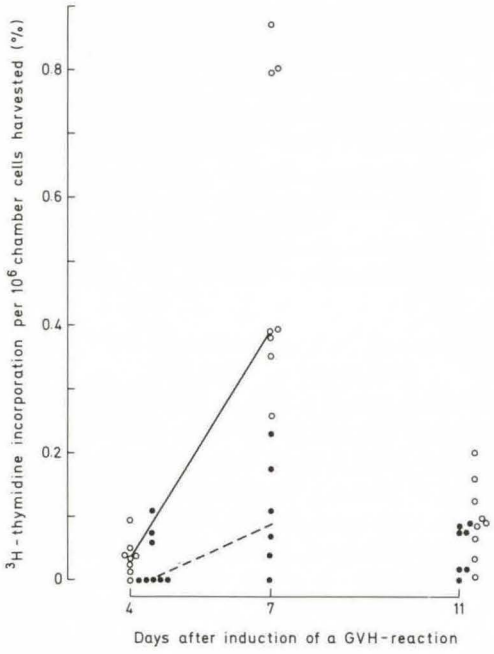


Fig. 1 The effect of an ongoing systemic GVH reaction on chamber cell proliferation. $^3$H-thymidine incorporation of cells cultured in GVH rats (o) compared with that in control rats (•). Chambers were implanted on the day of GVH induction, except results from day 11, where chambers were implanted 6 days after induction. Data are values from individual chambers, with lines connecting median values.

100 µl of Fischers medium containing 10 mmol/L Hepes buffer, 15 % (H0xDA)$F_1$ plasma and 200 µg/ml ampicillin were implanted into the peritoneal cavity of an $F_1$ host. After chamber retrieval, the cells were labelled in the chambers in vitro with $^3$H-thymidine (Code TRK 120, Amersham, England). $^3$H-activity per $10^6$ cells harvested was calculated as a percentage of the activity present in one ml of the incubation medium (1). The yield of cells harvested from the chambers was about 60–80 % of the cells inoculated and there was no statistically significant difference in yield between chambers from GVH hosts compared to control hosts.

4. *Injection schedule for preparing the GVH serum.* 3 male (HOxDA)$F_1$ rats were injected sc with 30 x $10^6$ HO TDL. The dose was divided between the 4 foot-pads (5 x $10^6$ cells in each) and the subcutaneous tissue of the back (10 x $10^6$ cells). One month later the same dose of cells was given sc, and in addition 30 x $10^6$ cells iv. The final boosting was carried out 4 months later with 50 x $10^6$ cells sc + 30 x $10^6$ cells iv. The rats were exsanguinated one week later, the sera pooled and heat inactivated. They were stored at –20 °C until use. As control serum served normal heat-inactivated $F_1$ serum.

5. *Preincubation of donor cells in GVH serum.* HO TDL (40–60 x $10^6$ cells/ml, vol. 1–2 ml) were incubated at 37 °C for 1–1 1/2 h and for further 1/2–1 h at room temperature before injection. In exp. 1 cells were incubated in one part of undiluted GVH serum (or alternatively, normal $F_1$ serum) + one part of complement (fresh undiluted unabsorbed guinea pig serum), washed and resuspended in the incubation medium before injection. In exp. 2 and 3 complement was omitted, the cells were incubated in undiluted serum and injected directly in their suspension medium. Viability, as judged by trypan blue exclusion, usually exceeded 95 %.

*Results*

1. *Cell proliferation in diffusion chambers in rats undergoing a systemic GVH reaction.* A dose of 200 x $10^6$ DA-TDL given iv to (HO x DA)$F_1$ hosts induced a vigorous systemic GVH reaction with lymphoid hyperplasia, dermatitis, runting and host death within 2–4 weeks. The spleen increased gradually in weight from about 0.4 g up to about 2 g on day 11 of the response. An increase in $^3$H-thymidine uptake of chamber cells in such hosts was also observed on day 7 of the response (Fig. 1). The ratio between median $^3$H-thymidine uptake of test and control cells (from animals injected with suspension medium only) was 4.4 in this experiment. In two similar experiments the ratios were 3.0 and 2.0, respectively. The latter difference was not statistically significant; however, when a two-sided Wilcoxon-van Elteren test (5) was applied to all 3 experiments (including a total of 19 chambers in the GVH group and 17 chambers in the control group), the stimulation of cell proliferation in chambers of GVH rats on day 7 was

highly significant ($p < 0.001$). Nevertheless the great majority of the chamber cells at harvest were small lymphocytes; 5–15 % were scored morphologically as activated cells in both the test and the control group.

2. *Effect of a GVH serum on the local popliteal lymph node GVH reaction.*

This serum was originally raised with the intention of producing an anti-receptor serum (17). It was therefore surprising that, even when the cells were incubated with serum and complement, the serum was found to augment, rather than depress, the GVH activity of the parental cells from the strain used to induce the formation of it (Table 1). However, complement was not necessary for the potentiating effect on lymph node enlargement (Table 1). Moreover, the serum was only active during an ongoing GVH reaction, since when injected alone, it caused no lymph node enlargement (Table 2).

Table 2 Effect of a GVH serum injected alone on the popliteal lymph node enlargement

| Lymph node weight (mg) | | Ratio |
|---|---|---|
| GVH serum | Normal $F_1$ serum | GVH/normal |
| 4.12 | 5.53 | 0.74 |
| 4.17 | 4.75 | 0.87 |
| 3.92 | 7.21 | 0.54 |
| 5.98 | 4.93 | 1.21 |
| Median ratio: | | 0.80 |

*Discussion*

During systemic as well as local GVH reactions, a burst of cell proliferation often precedes and accompanies the lymphoid hyperplasia (4, 9, 12, 19). Cellular proliferation thus seems to be an important pathogenic mechanism behind the increase in cellularity of the lymph nodes and spleen. These organs are also the sites of early donor cell localization (10). The donor cells could conceivably trigger host cell proliferation exclusively by cell contact or short range mediators. The present demonstration of an increased host cell proliferation in cell impermeable diffusion chambers gives evidence for another possible mechanism, namely that factors having the capacity of stimulating cell proliferation at a distant site, are released into the body fluids during GVH reactions.

The increase in $^3$H-thymidine uptake of chamber cells in GVH hosts by a factor of only 2–4, and the low proportion of activated

Table 1 Effect of preincubation of donoer cells with a GVH serum on the GVH activity of the cells, as measured by the popliteal lymph node response

| | Number of donor HO cells injected | Lymph node weight (mg) GVH serum | Normal $F_1$ serum | Ratio GVH/Normal |
|---|---|---|---|---|
| Exp. 1.[1] | $2 \times 10^6$ | 71.8 | 32.4 | 2.22 |
| | | 43.3 | 20.5 | 2.11 |
| | | 52.7 | 31.1 | 1.69 |
| | | 41.2 | 22.7 | 1.81 |
| Exp. 2.[2] | $3.6 \times 10^6$ | 22.6 | 18.2 | 1.24 |
| | | 32.2 | 11.2 | 2.88 |
| | | 10.3 | 9.2 | 1.11 |
| | | 21.3 | 7.0 | 3.02 |
| Exp. 3.[2] | $4.2 \times 10^6$ | 12.2 | 29.3 | 0.41 |
| | | 59.3 | 33.6 | 1.77 |
| | | 81.2 | 20.9 | 3.89 |
| | | 62.6 | 23.4 | 2.68 |
| Median ratio: | | | | 1.96 |

1) Cells preincubated with guinea pig complement + GVH serum.
2) Cells incubated with GVH serum alone.

cells found among the cells harvested, need not indicate that humoral factors only play a limited role in induced cell proliferation in the lymphoid tissues. During a local GVH-reaction in the popliteal lymph node, the proportion of S-phase cells only rose from 2 % up to a maximum of 6 % at the height of the response. Still, the nodes increased in weight by a factor of 10–15 (19). Furthermore, the early appearance of the mitogenic factors in the peritoneal fluid (well before the spleen hyperplasia had reached its maximum), suggests that they might be instrumental in the development of the lymphoid hyperplasia, and not just being secondary to an ongoing GVH reaction. However, the demonstration of mitogenic factors in the body fluids of rats undergoing a systemic GVH reaction might depend critically on the experimental conditions used for their detection. Though they were present in sufficient quantities to trigger chamber cell proliferation when (HOx DA)$F_1$ rats received DA cells, no increased cell proliferation could be demonstrated when the $F_1$ hybrids received identical numbers of HO cells (unpublished, preliminary data).

It is not clear how the GVH-serum potentiated the GVH-activity of the parental cells. Although the serum of the present studies was raised by multiple injections of parental cells into $F_1$ hybrids, other experiments have shown that a GVH serum with similar effect can be produced after only one single sc injection of parental cells in $F_1$ hybrids (3).

The most plausible action of the GVH-serum was on the mitotic activity of cells in the stimulated node, since a close correlation seems to exist between lymph node enlargement and degree of cell proliferation (6, 19). Other mechanisms, like trapping of lymphocytes from the circulation are probably less important (18). The GVH-serum factor and the mitogenic factor of the type detected in the diffusion chamber experiments could therefore possibly be similar or identical. Although the serum injected alone produced no lymph node enlargement, this does not necessarily indicate any fundamental differences between the two factors. Since the lymph flow dynamics in nodes undergoing a GVH reaction might be different from that in normal nodes, the exposure of the lymph node cells to humoral factors penetrating the node from the afferent lymphatics might also be unequal.

However, it is also conceivable that the two main phenomena of the present work are quite unrelated to each other. Moreover, the relationship between the present observations and the mitogenic factors released during allogenic cell contact in vitro (11, 14, 21) remains a matter of further investigation.

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*Bent Rolstad, M.D., Institute of Physiology and Anatomical Institute, University of Oslo (Karl Johansgt. 47), (Oslo 1, Norway)*