LYMPHOID TISSUE TRANSPLANTATION IN RATS LEADS TO A GVHR, INDUCING A SPECIFIC T-CELL MEDIATED AUTOREACTIVITY AGAINST MHC-ANTIGENS

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As a biology model for the analysis of GVHR, caused by continuously supplied immunocompetent cells of a lymphoid tissue rich organ, small intestine (SI) transplantation was performed in rats.

MATERIALS AND METHODS

Animals

Rats of the inbred strains LEW ($RT1^l$), BN ($RT1^n$), F344 ($RT1^{lvl}$), CAP ($RT1^c$) and their F_1-progeny were used; recipients at the age of 6, graft donors at the age of 3-4 months.

Technique of SI Transplantation

For transplantation of SI the so-called bypass technique was used, which consists of grafting the organ heterotopically as an auxilliary organ (Fig. 1): the aboral end of the graft was anastomozed in an end-to-side technique to the recipient's terminal ileum, while the oral end of the graft was closed with a circular ligature.

Test Procedure for Cell Mediated In Vitro Reactivity

Cell mediated in vitro reactivity of the various lymphatic compartments of the recipient (blood = bl, spleen = sp, peripheral

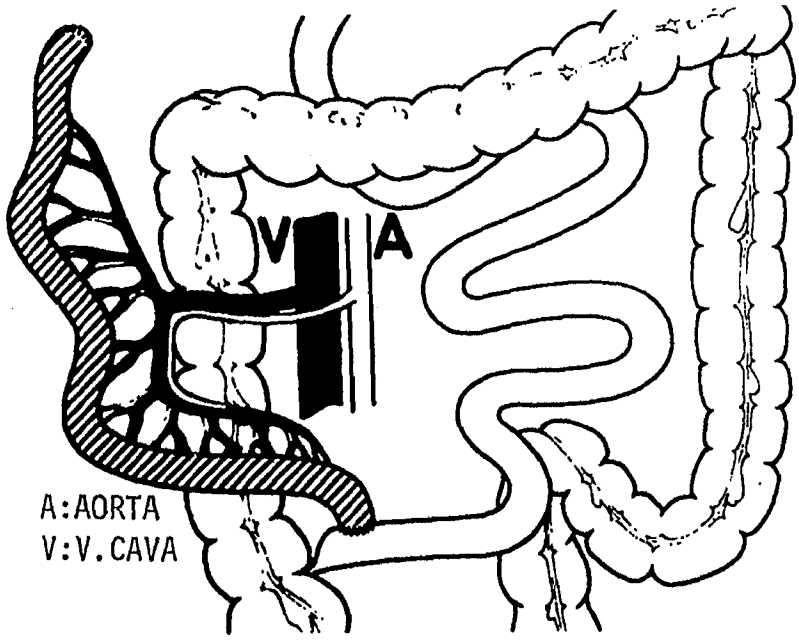

FIG. 1. Schematic outline of heterotopic small intestine transplantation.

lymph nodes = pln, mesenteric lymph nodes = mln, Peyer patches = pp) and of the graft (mesenteric lymph nodes = mln-gr, Peyer patches = pp-gr) was tested at different times (14-30, 50 or 90-110 days) p.op. using the microcytotoxicity assay, MCA (1). Embryonic fibroblasts of parental strains and a third party strain served as target cells.

Experimental Groups

Four experimental groups were formed, grafting either total of half SI of parental origin into F_1-hybrids I. 1/1 F344 → (F344 x CAP)F_1, II. 1/2 F344 → (F344 x CAP)F_1, III. 1/1 BN → (LEW x BN)F_1 and IV. 1/2 BN → (LEW x BN)F_1.

RESULTS

Mortality Rates

GVHR induced mortality of semi allogeneic graft recipients in the four experimental groups is shown in Fig. 2. As can be seen, mortality of SI recipients clearly depends on (a) the kind of strain combination used and (b) the amount of lymphatic tissue grafted (total or half SI).

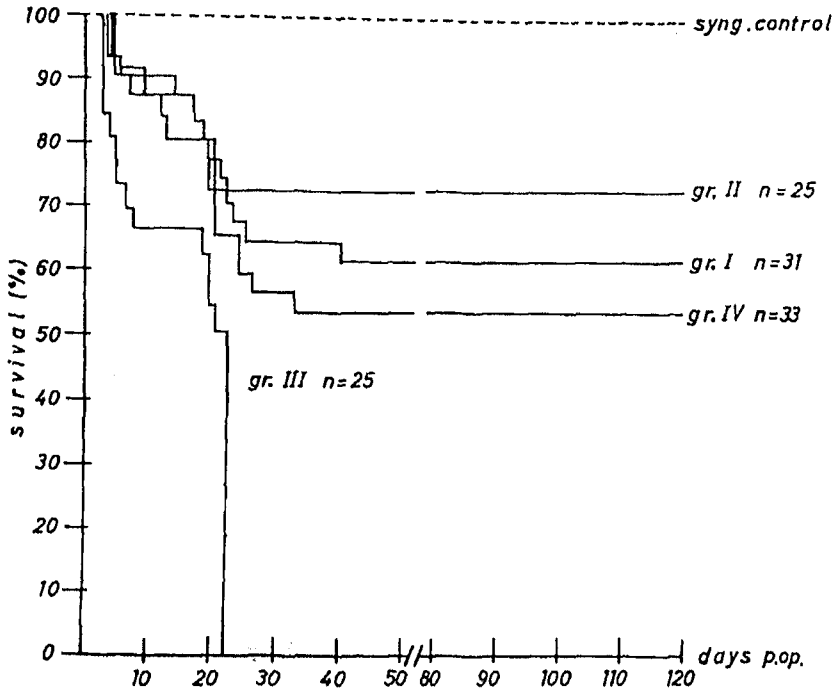

FIG. 2. Survival of small intestine graft recipients.

Characterization of GVHR

For measuring GVHR, embryonic fibroblasts from that parental strain which did not provide the graft, were used as targets. Figs. 3-5 demonstrate cell compartment, strain combination and serum factor dependence of in vitro measurable GVHR. With regard to cell compartment dependence, two main patterns have been observed: (a) lack of reactivity in the transport compartment blood combined with reactivity in the various lymphatic tissue compartments, interestingly including donor and recipient Peyer patches; (b) remarkable reactivity in the transport compartment blood combined with reactivity in the various lymphatic tissues, excluding donor and recipient Peyer patches. These strongly differing patterns appear to be correlated with the mortality rates in the strain combinations studied, in so far as GVH-reactivity in blood is observed in animals which die within the following week. On the other hand, long term survivors without blood compartment reactivity contain factors in their serum which are able to inhibit cell mediated reactivity (Fig. 5).

Characterization of "Self-Constituents-reactivity" (SCR)

For measuring SCR, embryonic fibroblasts from that parental strain which did provide the graft, were used as target cells. As evident from Figs. 3-5 the observed SCR follows the same pattern

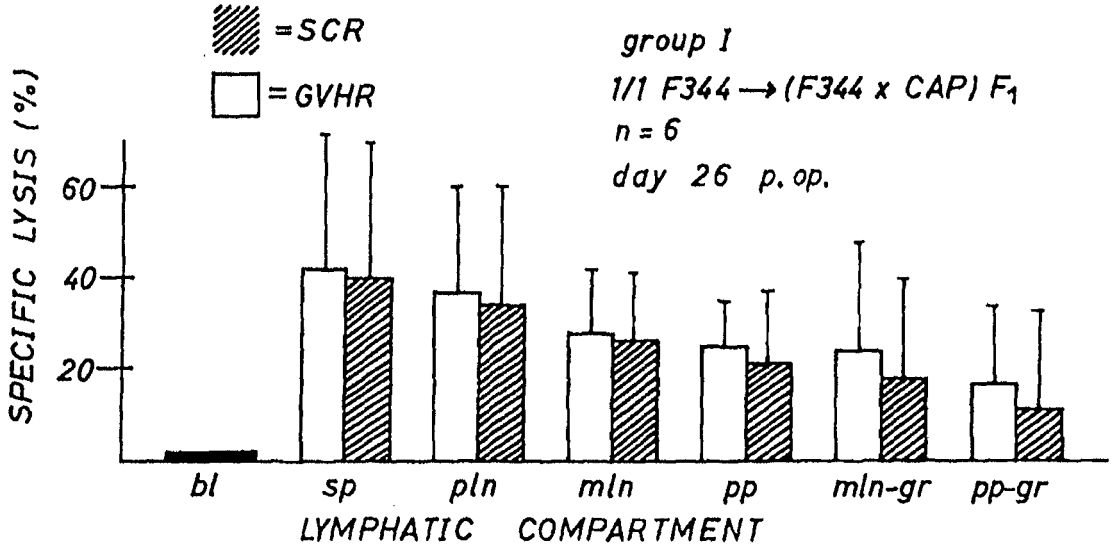

FIG. 3. Cell mediated in vitro reactivity of the various lymphatic compartments of the recipient and of the graft in group I.

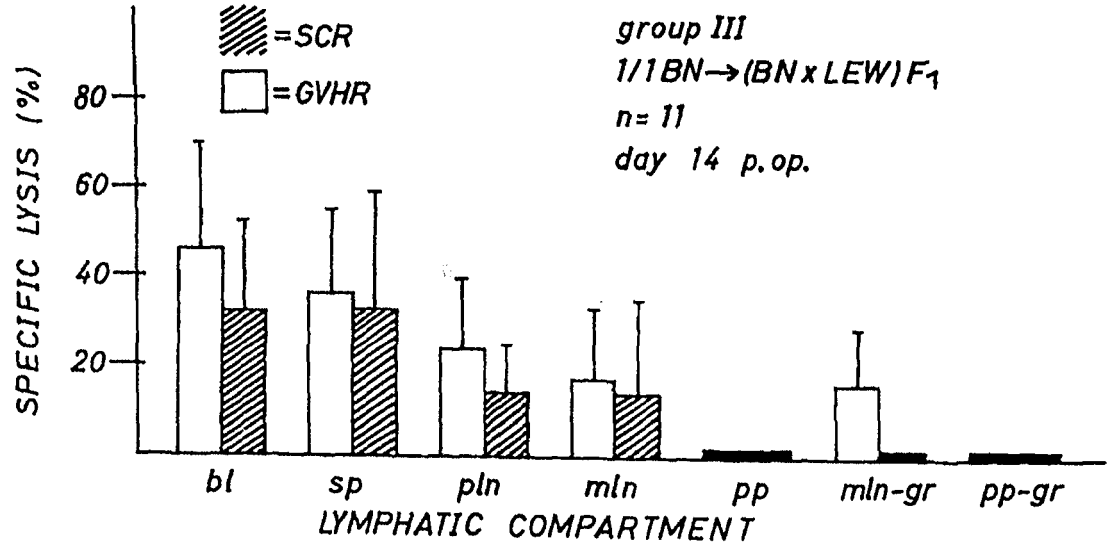

FIG. 4. Cell mediated in vitro reactivity of the various lymphatic compartments of the recipient and of the graft in group III.

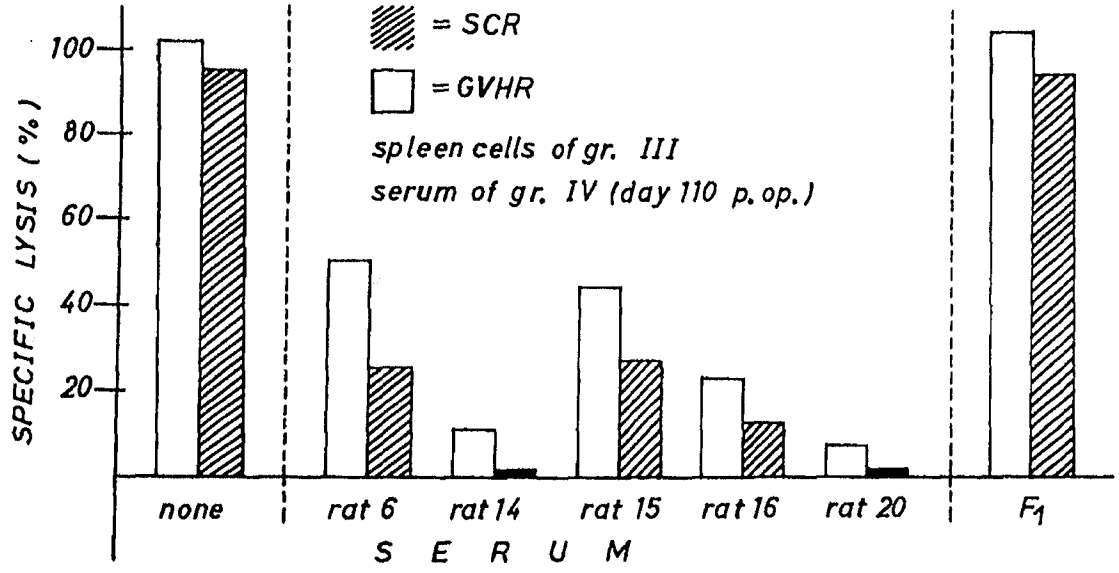

FIG. 5. Inhibition of cell mediated in vitro reactivity by serum.

of appearance as that of GVHR, i.e., it shows cell compartment, strain combination and serum factor dependence. However, though dependent on GVHR, it follows independent kinetics, when measuring both reactivities on day 25, 50 and 110 p. op. (data not shown in Figs.).

Further Characterization of Reactive Cells and Targets

Reactive spleen cells were shown to be SAL sensitive, that is sensitive against specified antilymphocyte serum, which is exhaustively macrophage absorbed (2). Furthermore, no reactivity was observed when using third party target fibroblasts; that is, both GVHR and SCR appeared to be specific.

DISCUSSION

Two strain combinations strongly differing in GVHR induced mortality have been investigated. From the findings in Fig. 2, we conclude that mortality differences between the two strain combinations are hardly impressive when grafting only half SI, but quite impressive when grafting total SI. Strong differences occur even within one strain combination when grafting half vs. total SI. Considering that the F344/CAP combination is characterized by

3 but the LEW/BN combination by 5 MHC mismatches, this difference in histoincompatibility, obviously fully effective only in full length grafts, could be regarded as a reasonable explanation for the differences in mortality.

The most interesting finding concerning GVHR after SI transplantation, namely, its compartmentalization, allows for the hypothesis that lymphocytes may become the more aggressive to the host, the better they are able to move free in the blood. Once rats have survived the GVH-crisis between day 10 and 20, cytotoxic effector cells are missing in the transport compartment blood and appear to be "locked in" into the lymphoid tissue compartments. A similar "locking-in phenomenon" (nonreactivity in blood vs. reactivity in the splenic compartment) has been observed several years ago in rats specifically tolerating skin allografts (3). Additionally to this cell mediated phenomenon, serum factors become demonstrable which block cytotoxic effector cells in vitro. How far these two phenomena can be correlated and which the nature of the serum factor(s) is, awaits clarification.

Autoantibodies, elicited by GVHR, were observed some years ago by Gleichmann and Gleichmann (4). On the other hand, an in vitro activation of F_1-hybrid cytotoxic T-lymphocytes specific for self H-2 has recently been described by the Cudkowicz group (5). The findings described here may be considered in this context and, even, regarded as a connecting link in that in vivo (rather than under the more or less artificial in vitro conditions) developing cell mediated autocytotoxicity (rather than auto-antibody production) has been observed. Concerning mortality, when SCR as well as GVHR can be demonstrated in the transport compartment, the question must be raised whether it is SCR rather than GVHR which kills the graft recipients.

A number of questions call for further investigation: (a) What is the nature of the autoreactive cell? (b) From where does the autoreactive cell originate (Nakano et al. (5) found the F_1-hybrid cell to be reactive)? (c) What is the meaning of the independent kinetics of GVHR and SCR? (d) How far are both reactivities determined by the continuous supply of immunocompetent cells after SI transplantation?

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