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***In vivo* and *in vitro* effects of anti-idiotypic auto-immunization in the rat,** par B. CHARPENTIER, Ph. LANG, Bernadette MARTIN, O. ESPINOSA, D. FRIES, *Laboratoire de Transplantation d'Organes, Service de Néphrologie, Université Paris-Sud, Hôpital Paul Brousse, 94800 Villejuif, France.*

It has been reported that transplantation tolerance can be achieved in rodents by using anti-idiotypic antibodies or autoimmunization with mixed lymphocyte culture (MLC)-generated T blasts (Andersson *et al.*, 1977). Both methods lead to the specific elimination of T cell clones bearing receptors for alloimmune determinants. The aim of this study was to investigate the effects of anti-idiotypic autoimmunization in inbred rat strains.

Inbred male rats were used : Brown-Norway (BN) ($RT1^n$), Lewis (lew) (RT^l), LBN (F_1) hybrid, DA ($RT1^a$), BNDA (F_1) hybrid, LDA (F_1) hybrid.The generation of large quantities of specific lymphoblasts, lymphoblast purification and animal immunization with lymphoblasts have already been described (Charpentier *et al.*, 1979). In brief, lymphoblasts were obtained *in vitro* in a macro-MLC and further purified on 1-g velocity sedimentation. Animals were injected IP with Freund's adjuvant at 2-week intervals with 10^7 lymphoblasts. MLC test : PBL and/or spleen cells were cultured with 1500 R-irradiated stimulatory cells for 5 days at 37 °C in flat-bottomed tissue culture microplates. The cultures were labelled with ^{3}H-TDR and harvested with a MASH II apparatus. Cell-mediated lympholysis (CML) assay : effector lymphocytes were obtained from the spleen of autoimmunized and control animals. The targets were either PHA blasts or MLC-generated lymphoblasts labelled with ^{51}Cr and mixed with various amounts of effector cells in culture microplates. After 4-h incubation, 100 μl of supernatant was pipetted off and radioactivity was measured in a γ counter. The percentage of specific lysis was determined according to the usual formula :

$$\frac{\text{experimental release (cpm)} - \text{control release (cpm)}}{\text{maximal release (cpm)} - \text{control release (cpm)}} \times 100$$

Heterotopic cardiac grafts were carried out intrabdominally according to the usual microsurgical method. When ventricular contractions were no longer present, the graft was considered as rejected.

The survival of semi-allogeneic heart grafted into untreated lew was 9.3 $\pm$ 1.6 days in a group of 25 control animals. Heart prolongation in lew sensitized IV once with 10^7 blasts without Freund's adjuvant was not different from that of the controls. On the other hand, it was necessary to use three consecutive IP injections of 1 $\times$ 10^7 blasts each plus Freund's adjuvant in order to obtain evidence of anti-idiotypic antibodies and/or of specific unresponsiveness in MLC against the relevant stimulatory strain. These lew rats, apparently unresponsive to lew $\times$ BN, were heart-transplated. None of the 16 *in vitro* tolerant rats showed different survival from that of the controls. All autoimmunized rats were tested for allogeneic recognition with MLC assay and for anti-idiotypic antibodies in a MLC-blocking assay. Peripheral blood lymphocytes (PBL), obtained by bleeding from the orbital venous plexus, were tested 5 and 21 days aftet the last IP injection in parallel with normal control lymphocytes. In a group of 33 rats autoimmunized IP three times with 1 $\times$ 10^7 blasts at 2-week intervals only 10 evidenced a loss of lymphocyte reactivity in MLC. This unresponsiveness was chiefly observed 3 weeks after the last booster before transplantation. These rats gave a proliferative response 20 % lower than the normal lew response. Spleen cells from autoimmunized rats were also tested for the generation of cytotoxic T cells in a 5-day MLC against lew $\times$ BN spleen cells of lew $\times$ DA spleen cells. Five rats in the group of 10 unresponsive ones in MLC were tested for their ability to generate CTL. None of the 5 rats generated CTL against lew $\times$ BN spleen cells, whereas they did against the irrelevant lew $\times$ DA strain.

These experiments show that specific unresponsiveness in MLC and in CML could be obtained after anti-idiotypic autoimmunization with MLC-generated blasts in rats. But heart

transplantation tolerance was never observed in this group of apparently *in vitro* tolerant rats. In our conditions, three consecutive IP injections of 1×10^7 blast cells with Freund's adjuvant were necessary to obtain *in vitro* loss of alloreactivity. Our data are at variance with those obtained after heart grafting in other strains of rats (Aguet *et al.,* 1978 ; Hayry *et al.,* 1981).

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