In vivo and in vitro effects of anti-idiotypic auto-immunization in the rat. par B. CHARPENTIER, Ph. LANG, Bernadette MARTIN, O. ESPIÑOSA, 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) (RT1a), Lewis (lew) (RT1b), LBN (F1 hybrid), DA (RT1a), BNDA (F1 hybrid), LDA (F1 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 3H-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 51Cr 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:

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\text{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.}
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The survival of semi-allogeneic heart grafted into untreated lew was 9.3 ± 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 x 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 x BN, were heart-transplanted. 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 after the last IP injection in parallel with normal control lymphocytes. In a group of 33 rats autoimmunized IP three times with 1 x 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 x BN spleen cells of lew x 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 x BN spleen cells, whereas they did against the irrelevant lew x 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 $1 \times 10^7$ 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).


