Prolonged Survival Time of Allogeneic Skin Grafts in Host Pretreated with Somatic Cell Hybrids and Prednisolone

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NISHIHIRA, T., ISHII, H., KASAI, M. and TACHIBANA, T. *Prolonged Survival Time of Allogeneic Skin Grafts in Host Pretreated with Somatic Cell Hybrids and Prednisolone.* Tohoku J. exp. Med., 1979, 129 (1), 71-74 — By applying somatic cell hybrids to transplantation immunity, the prolongation of survival time of mouse skin allografts was investigated. The hybrid cells (LJ) were made by cell fusion between L cells derived from C3H/He mouse and JLS-V9 cells derived from BALB/c mouse with UV-irradiated Sendai viruses. Skin grafts from female (C3H/He-Jms × BALB/c-Slc) F₁ donors were transplanted to male BALB/c-Slc. The survival of the transplant in BALB/c pretreated with the LJ cells and prednisolone showed a significant prolongation, compared with that in animals pretreated with only prednisolone or the LJ cells. The mean survival time and standard deviation of the grafts in mice injected with 1×10^5 LJ cells i.v. 4 days before the grafting, and subsequently treated with prednisolone 50 mg/kg i.p. 1 day before and at the time of the grafting were 17.3 ± 2.2 days. Controls were 9.2 ± 0.4 days. The mechanisms of prolongation of skin grafts were discussed in terms of cell-mediated immunity and humoral factors. Somatic cell hybrids are useful, not only for inducing immunological enhancement, but also for studying the function of major histocompatibility antigens in cellular immunity and can be applied to clinical organ transplantation. — somatic cell hybrids; prolongation of skin grafts; immunological enhancement; selective hyporesponsiveness

Somatic cell hybridization has recently been applied to the induction of tumor immunity (Watkins and Chen 1969; Tachibana et al. 1974), in addition to its usefulness for the analyses of the location of gene loci on human chromosomes. It has been generally demonstrated that somatic cell hybrids express codominantly the surface antigens which are introduced by both parental cells (Spencer et al. 1964;

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Silagi 1967). Also, it has been reported that the growth of some somatic cell hybrids of tumor cells with normal cells is suppressed even in normal syngeneic host and that they concomitantly induce the resistance of the host to the challenge of parental tumor cells (Tachibana et al. 1974; Liang and Cohen 1976). It means that somatic cell hybrids can evoke an efficient T cell reactivity in the normal host.

Thus, the clonal expansion of immune cells specifically reactive to allogeneic antigens can be induced by the inoculation of somatic cell hybrids. This clonal expansion may be reduced by the subsequent administration of immunosuppressant. But, the antibody produced may remain after immunosuppression and would be expected to work on as an enhancing antibody. It is possible, therefore, that the inoculation of somatic cell hybrids followed by immunosuppressant would bring about beneficial effects on the survival of grafting tissue.

We have attempted to apply somatic cell hybridization to the research on transplantation immunology. This is the first report concerning the survival effect on skin grafting based on the combination of inoculation of somatic cell hybrids with subsequent administration of immunosuppressive drug.

MATERIALS AND METHODS

8-azaguanine resistant L cells derived from C3H/He mouse (H-2^k) were fused with 5-bromodeoxyuridine resistant JLS-V9 cells derived from BALB/c mouse (H-2^d) by using UV-irradiated Sendai viruses (Murayama-Okabayashi et al. 1971). The hybrid cells (LJ) were selected in HAT medium. The LJ hybrids were maintained in Eagle's MEM medium supplemented with 10% calf serum and expressed both parental H-2 haplotypes (H-2^{d/k}).

The recipients were injected with 1×10^5 LJ cells i.v. 4 days before the grafting, and treated with prednisolone 50 mg/kg i.p. 1 day before and at the time of the grafting. Full thickness skin grafts from female (C3H/He·Jms $\times$ BALB/c·Slc)F₁ donors were transplanted to the back of male BALB/c·Slc mice. Ten to 20 mice were used as an experimental or a control group.

One way mixed lymphocyte reaction (MLR) was performed with peripheral blood lymphocytes (PBL) obtained from axillary arteries (Nishihira and Fujii 1972). Stimulating cells of F₁ hybrid PBL were irradiated with 1,000 rads electron beam and those of LJ cells with 8,000 rads.

RESULTS

The survival of the transplant in BALB/c mice treated with prednisolone and the LJ cells showed a significant prolongation, compared with that in animals treated with only prednisolone or the LJ cells.

The mean survival time and the standard deviation of the grafts are shown in Table 1. The combination of F₁ hybrid spleen cells or bone marrow cells carrying the same H-2^{d/k} haplotypes and prednisolone did not induce any significant prolongation of the grafts.

One way MLR between F₁ mice and the recipients with prolonged skin graft was lower than the MLR between F₁ mice and normal BALB/c mice. The sera of the recipients with prolonged skin graft inhibited the MLR between F₁ mice and normal BALB/c mice, suggesting the presence of blocking factors. The LJ cells

TABLE 1. *Survival times of skin allografts ((C3H/He × BALB/c) F₁ → BALB/c)*

Pretreatment of recipients with	Number of mice	Survival time of the grafts (mean ± s.d.)	Difference from control
LJ hybrids, 1×10^6 cells i.v. (-4 day)	11	9.3 ± 1.0	$p < 0.1$
Prednisolone, 1 mg/20 g i.p. (-1 and 0 days)	10	9.9 ± 1.3	$p < 0.1$
LJ hybrids, 1×10^6 cells i.v. (-4 day) and prednisolone, 1 mg/20 g i.p. (-1 and 0 days)	10	17.3 ± 2.2	$p < 0.01$

irradiated with 8,000 rads stimulated more strongly BALB/c lymphocytes in MLR than F₁ hybrid spleen cells or bone marrow cells as stimulator cells.

DISCUSSION

Transplantation immunology has clarified that the specificity of the antigens involved in graft rejection is under genetic control. Of many gene loci controlling these antigens, one complex locus termed the major histocompatibility complex (MHC) controls the strong transplantation antigens such as H-2 antigens in mouse and HLA antigens in man which provoke intense allograft reactions. Therefore, it is reasonable that allograft rejection would be prevented by matching donor and recipient at the MHC locus. But, it is practically difficult to obtain an identical matching of the MHC. Thus, general immunosuppression and immunological enhancement are considered as other possibilities for the prevention of graft rejection. However, the induction of immunological enhancement of the graft has not even now been successful, unlike such an effect on survival of tumor allografts. Recently, another possibility has been suggested that autoantidiotypic immunity after antigen-specific blast immunization could be useful for survival of grafts by the induction of specific reduction in T cell reactivity against the relevant alloantigens (Aguet et al. 1978).

Our preliminary results described here suggest the possibilities of the induction of enhancement phenomenon as well as a presumably selective decrease of T cells reactive to the relevant alloantigens in addition to general immunosuppression by the pretreatment of the recipients with the combination of somatic cell hybrid and immunosuppressant. The problems concerning doses of immunization cells, times of injection and programming of the combination of the hybrid cells with immunosuppressant still remain to be solved. Further investigations of the mechanisms on prolonged survival of graft are in progress.

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