Introduction: A number of experimental studies have proven the need for long term systemic administration of immunosuppressants to the recipient for successful peripheral nerve allografts. Therefore, the undesirable side effects, such as opportunistic infection, renal failure and malignancy, due to the immunosuppressants should be seriously considered. To prevent these side effects, new immunosuppressive strategies must be established. Recent studies have described that T cell activation requires two signals. First signals which occurs in the interaction of the T cell receptor with its specific peptide bound to major histocompatibility complex, can be prevented using immunosuppressive drugs, such as cyclosporine and FK506. Second (costimulatory) signals are provided by the ligation of cell adhesive molecules on the T cell and their ligand counterpart on the antigen-presenting cell (APC). CTLA4Ig, which is a soluble recombinant fusion protein, binds B7 with high affinity, and can block the costimulatory signal needed for optimal T cell activation. Several experimental studies have shown that CTLA4Ig can prevent acute allograft rejection without serious side effects.

In the current study, we hypothesized that the technique of gene transduction could induce the local expression of CTLA4Ig in nerve allografts. The objectives of this study were to clarify the following using rat models: (1) Can the CTLA4Ig be expressed by local administration of the adenovirus containing CTLA4Ig-gene (AdexCTLA4Ig) in nerve allografts? and (2) Is the acute rejection of the allograft prevented by the local administration of AdexCTLA4Ig?

Method: We have constructed a recombinant adenovirus, AdexCTLA4Ig, containing the extracellular domain of murine CTLA4 and the Fc portion of human immunoglobulin G1 under the control of the CAG promoter, which is composed of the cytomegalovirus enhancer and chicken β-actin promoter. This experimental protocols were approved by the Committee on Animal Experimentation of Hokkaido University. To confirm the expression of CTLA4Ig molecules, AdexCTLA4Ig (3 × 10⁸ pfu/ml) was intravenously administered to rats (n=4). At 7 and 14 days after the administration, rats were sacrificed to obtain for immunohistochemical study.

ACI rats were used as donors and Lewis rats were used as recipients. As serving a nerve allograft model, a 1.5cm segment of sciatic nerve of ACI rats was transplanted to Lewis rats. The recipient rats were divided into three groups as follows; Group1 (n=4), syngeneic graft (Lewis to Lewis); Group2 (n=4), allogeneic graft without immunosuppressions; Group3 (n=8): allogeneic graft with the local injection of AxCTLA4Ig (3 × 10⁵ pfu/ml) to the grafted nerve at the time of operation. At 7 and 14 days after transplantation, the two grafts in Group3 were removed for immunohistochemical staining to detect the local expression of CTLA4Ig. For the histological evaluations of the grafts, four grafts in each group were obtained at 14 days postoperatively. The graft rejection was quantitated using the standard histological grading system (0 to 6: no rejection to severe rejection). The mean histological grading score (HGS) for each group was statistically compared by ANOVA. P values less than 0.05 were considered significant.

Results: 1) At both 7 days and 14 days after venous administration of AdexCTLA4Ig, the expression of CTLA4Ig in hepatocytes was detected (Fig.1). 2) While the local expression of CTLA4Ig in the grafted nerve was identified at 7 days after operation (Fig.2), there was no local expression at 14 days. In histological evaluation, the grafts in Group 2 showed severe rejection with the increase of connective tissue and the infiltration of mononuclear cells. In Group 3, grafts showed mild rejection. The mean HGS in each group was 1.0 in Group 1, 4.5 in Group 2, and 3.1 in Group 3 (Fig.3). The score in Group 3 was significantly lower as compared with that of Group 2 and 3.

Discussion: The current study demonstrated the local expression of CTLA4Ig in the nerve allografts using adenovirus vector. In addition, the efficacy of this fusion protein against the allograft rejection was proved. Although long-term graft acceptance was not achieved by the current protocols, our results support the feasibility of gene therapy for local immunosuppression using CTLA4Ig in allogeneic nerve grafts. We believe that local administration of AdexCTLA4Ig will overcome the current problems in allogeneic nerve graft mentioned above.

Fig.1 Immunohistochemical staining of CTLA4IgG, using anti human immunoglobulin G antibody, was performed on the liver tissue derived from Lewis rats on day 14 after administration of AdexCTLA4Ig. The expression of CTLA4Ig was found in hepatocytes (×200).

Fig.2 Immunohistochemical staining of the grafted nerve on day 7 after transplantation. The specimen showed the findings indicating the expression of AdexCTLA4Ig in Schwann cells(×100).

Fig.3 Histological grading score (HGS) on day 14 day after transplantation. (mean ± SE) * P<0.05 compare with Group 2. Mean HGS of Group1 is statistically significantly lower than that of Group 2 and 3.

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