Introduction: A number of experimental studies have proven the need for long-term administration of immunosuppressants to recipients in order to ensure for successful peripheral nerve allografts. Therefore, the undesirable side effects of the immunosuppressants should be 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 occur in the interaction of the T cell receptor with its specific peptide bound to the major histocompatibility complex, can be prevented using immunosuppressive drugs, such as cyclosporine A 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). CTLA4 Ig, which is a soluble recombinant fusion protein, binds B7 with high affinity, and can block the costimulatory signal required for optimal T cell activation. Several experimental studies have shown that CTLA4 Ig can prevent allograft rejection without serious side effects. In the current study, we hypothesized that the gene transfection using the adenovirus vector containing CTLA4 Ig-gene (AdCTLA4 Ig) could suppress acute rejection in peripheral nerve allografts. The objective of this study was to clarify the followings using rat models: (1) Is the acute rejection of the allograft prevented by the systemic and local administration of AdCTLA4 Ig? (2) Are there any differences in immunosuppressive efficacy between these administrations?

Method: We constructed a recombinant adenovirus, AdCTLA4 Ig, containing the extracellular domain of murine CTLA4 Ig and the Fc portion of human immunoglobulin G1 under the control of a CAG promoter, which is composed of a cytomegalovirus enhancer and a chicken β-actin promoter. These experimental protocols were approved by the Committee for Animal Experimentation at Hokkaido University. To confirm the expression of CTLA4 Ig molecules, AdCTLA4 Ig (3 x 10^9 pfu/ml) was intravenously administered to rats (n=5). Furthermore, the serum concentration of CTLA4 Ig was measured periodically by ELISA. ACI rats were used as donors and Lewis rats were used as recipients. Serving as a nerve allograft model, a 1.5 cm segment of the sciatic nerve of ACI rats was transplanted to Lewis rats. The recipient rats were divided into four groups as follows; syngeneic group (n=10), allogeneic graft without any immunosuppressions; systemic treatment group (n=9), allogeneic graft with systemic administration of AdCTLA4 Ig (1 x 10^10 pfu/ml) immediately after the operation; local treatment group (n=9), allogeneic graft with the local injection of AdCTLA4 Ig (3 x 10^9 pfu/ml) to the grafted nerve at the time of operation. At 7 and 14 days after transplantation, two liver tissues in the systemic treatment group, and the two grafts in the local treatment group were removed for immunohistochemical staining to detect the expression of CTLA4 Ig. For the histological evaluations of the grafts, five grafts in each group were obtained at 14 days and 8 weeks postoperatively. Graft rejection was quantitated using the standard histological grading system (0 to 6: no rejection to severe rejection). At 8 weeks postoperatively, motor nerve conduction velocity (MNCV) of the sciatic nerve across the nerve graft was measured. Statistical comparisons were performed using one-way ANOVA. Differences were considered significant for p<0.05.

Results: Expression of CTLA4 Ig: At both 7 and 14 days after venous administration of AdCTLA4 Ig, the expression of CTLA4 Ig in the hepatocytes was detected. The maximum serum concentration of CTLA4 Ig was obtained at 7 days after administration of AdCTLA4 Ig, then the concentration decreased gradually. The local expression of CTLA4 Ig in the grafted nerve was identified at only 7 days after operation.

Histological evaluation of allografts: The syngeneic group showed minimal cellular infiltration around the grafts with normal perineural structure after both 14 days and 8 weeks postoperatively. Excellent regeneration with well-myelinated fibers was also found in the grafted nerve at 8 weeks after operation (Fig.1A). By contrast, the grafts in the no treatment group showed severe rejection with an increase of connective tissue and the infiltration of mononuclear cells at 14 days postoperatively. The progression of these findings was found at 8 weeks after transplantation (Fig.1B). On the other hand, the grafts from the systemic treatment group showed mild rejection with no progression from 14 days to 8 weeks postoperatively (Fig.1C). Although the grafts from the local treatment group showed relatively mild rejection at 14 days, the grafts at 8 weeks showed severe rejection with the destruction of perineural structure (Fig.1D). The mean histological grading score (HGS) at 14 days was 1.0±0.0 (S.D.) in the syngeneic group, 4.5±0.4 in the no treatment group, 1.6±0.6 in the systemic treatment group, and 3.1±0.8 in the local treatment group. The scores in the systemic and local treatment groups were significantly lower as compared to that in the no treatment group (P<0.005). The mean MNCV at 8 weeks after transplantation was 0.1±0.2 in the syngeneic group, 4.5±1.2 in the no treatment group, 2.8±1.0 in the systemic treatment group, and 4.4±0.6 in the local treatment group. Although there was no statistically significant difference between the no treatment and the local treatment group, the value in the systemic treatment group was significantly lower than that in the no treatment group (P<0.005).

Discussion & Conclusion: This study demonstrated the efficacy of AdCTLA4 Ig against peripheral nerve allograft rejection. Based on the current data, central immunosuppressive actions by the systemic administration of AdCTLA4 Ig may be considered for immunosuppression against nerve allograft rejection. However, with efforts toward increasing the local expression of CTLA4 Ig in the grafted nerve, there is potential for achieving long-term graft acceptance without serious side effects.