JSPS Fellowship Accomplishment Report
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To: President
Japan Society for the Promotion of Science

Dear Mr. President:

It was a great honor to be awarded a "JSPS Invitation Fellowship for Research in Japan (Short-Term)" which allowed me, for the first time, to stay in Japan for one month (April 5 to May 5, 2014) to deeply involve in the medical research carried out in your country.

I was invited by Dr. Xiao-Kang Li, Chief, Division of Transplantation Immunology, National Research Institute for Child Health and Development at Tokyo to explore possible collaboration research projects based on our common interests, and compensated experiences.

During this short period of time, I accomplished the following research activities.

1. To attend the conference and deliver scientific lectures.
I attended the 12th International Society for Experimental Microsurgery Congress in Kyoto, Japan (April 11-13, 2014), and delivered a Keynote Speech entitled "Liver Transplant Tolerance – the History and Future" on April 12, 2014.
I have long been interested in development of clinically practical approach towards induction of transplantation tolerance.

I gave a lecture entitled "Cellular and Molecular Mechanisms in Liver Transplant

Tolerance" at the host Division of Transplantation Immunology, National Research Institute for Child Health and Development at Tokyo on April 17, 2014.

2. To discuss the potential collaborative research project (s) in Japan

I have been working on transplantation tolerance for many years, because organ transplantation is dependent on chronic administration of immunosuppression to non-specifically suppress host immune response, which is associated with multiple severe side effects. It has been a dream for transplant surgeons to achieve transplant tolerance without requirement of immunosuppression.

This may, indeed, not be a dream! Because transplantation tolerance occurs naturally after liver transplantation, which was first recognized by a group of surgeons in Europe when they practiced liver transplantation in farm pigs in early 1960's, and found that about 1/4 of liver allografts were not rejected. Similar observations were later reproduced in rats and mice. Interestingly, in human, also about 1/4 liver transplant patients can be totally withdrawn immunosuppression, which is not seen in other organ transplantation.

Can we identify these ¼ potentially tolerant patients before liver transplantation? This was the first research project I discussed with Dr. Xiao-Kang Li during this visit. Dr. Li and his colleagues have performed the genetic analyses for the patient following liver transplantation. However, the studies have focused on the recipients, as many other scientists.

I have also focused on studying the role of host immune response in liver transplant tolerance, and thought that the liver allograft might act as a thymus to be able to deplete the T cell clones that recognize allo-antigens. It turned out that T cell isolated from the recipient bearing long-term surviving liver allograft normally respond to donor antigens in vitro, demonstrating a "split" or operational tolerance phenomenon, indicating that host immune response is normal.

I was inspired by an elegant observation - liver allografts that deficiency in expression of interferon gamma receptor (IFN-γR) were all acutely rejected, implicating that lacking of an inflammatory receptor gens in graft can totally changes the outcome of transplant tolerance. I realized that we should focus on the graft. We have obtained convincing evidence demonstrating that liver transplant tolerance is actually mediated by the graft. The alloreactive response triggers the IFN-γR signaling in the graft tissue non-parenchymal cells, and promotes their expression of B7-H1, a T cell inhibitory molecule to control host immune response. Therefore, we should focus on the role of graft in induction of transplantation tolerance.

Most of liver transplantation in the US used cadaver livers, while a large port of liver transplants in Japan were living related, therefore, the donor samples are readily available. Dr. Li and I expressed common interests in this project, and agreed to initiate is as soon as possible. We will outline a plan in detail, and submit to IRB for approval. We will explore the research funding in the US and Japan, respectively, to support this project.

3. To enhance ongoing research collaborations.

It is my pleasure to recognize that these collaborative research projects have been well conducted in the host institute, including differentiation of dendritic cells from iPS cells by comparison with the bone marrow derived-dendritic cells. We also reported our results regarding differentiation of myeloid-derived suppressor cells (MDSC) from iPS cells. We shared our experience and optimized the culture conditions.

I would like to thank Dr. Li for his efforts in arrangement of my visit. Following careful analysis of the experiment results and extensive discussions.

Abstract of my lectures in Japan.

Liver tolerance was initially described in 1960' by the surgeons who practiced liver transplantation in farmer pigs, achieving 23% spontaneous long-term survive, which was later reproduced in rats and mice. Of 461 liver transplant patients worldwide undergone immunosuppression weaning, 100 were completely free of immunosuppression, suggesting that ~22% of liver transplants are tolerant, similar to pigs. We established a mouse liver transplant model. In B10 to C3H combination, liver grafts are spontaneously accepted. This is not due to clonal deletion of the specific T cells, because the T cells from the recipients vigorously responded to donor antigens in vitro. The mechanisms underlying this 'split' or 'operational' tolerance remain incompletely understood. The transplanted liver grafts initially underwent heavy graft infiltration, but decreased spontaneously in a few weeks, associated with apoptosis of infiltrating cells. We were inspired by the liver allograft being acutely rejected in IFN-γ knockout recipients, which was confirmed by rejection of IFN-γ receptor (R)1$^{-/-}$ liver allograft in WT recipients, associated with attenuated apoptosis of the graft infiltrating cells. We isolated the graft non-parenchymal cells (GNHC), and found that almost all graft CD45^{+} cells were quickly become recipient origin (IFN-γR1^{+}), while CD45^{-} cells remain donor type (IFN-γR1^{-}), indicating a critical role of the graft non-hematopoietic cells. These graft CD45^{-} cells suppressed T cell response in MLR. Liver endothelial cells (LEC) have been shown immune-regulatory activity. Co-transplantation with LEC slightly prolonged islet allografts, compared to hepatic stellate cells (HSC) which protected islet allografts long-term, associated with elimination of effector T cells, accumulation of MDSC and Tregs. IFN-γR1$^{-/-}$

HSC failed to protect islets. IFN-γ stimulated B7-H1 expression on HSC. B7-H1$^{-/-}$ HSC lost ability to protect islet graft. In conclusion, Liver transplant tolerance absolutely requires inflammatory stimulation to GNHC, particularly HSC, which regulate immune response via IFN-γ/B7-H1 signaling pathway.