Transplantation Medicine -Overview from my works-

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Introduction

In recent years low invasive treatment attracts attention, but it is a common practice that the most important thing stays the same in surgical operations to precisely judge each degree of invasion. The condition of patients after organ transplantation is more complicated than that of the normal surgery. There is a burdened pathologic point for the outbreak of infectious disease occurring due to immunoreaction mainly derived from rejection and excessive control.

The author has learnt transplant immunology and research field called clinical reactions of immunosuppressive agents in the dawn of organ regenerative therapy in Japan. And the practical immunology essential for organ transplantation was found to be merely necessary for the cure for infectious disease, which is applicable not only for conventional immunology, but also for infectious disease and its treatment as prognosis. It is indispensable to grasp the overall knowledge in order to understand latest basic information about transplantation in respective organs. Furthermore, without understanding the history of clinical practices, up-to-date basic knowledge won't be applicable for clinical application even though rapidly-developing new knowledge is acquired.

In this summary, the outline for basics about allo (among different genealogy) immunoreaction based on immunological rejection. In the consequence, it refers to currently in use the point of action in molecule level of immunosuppressant with figures attached. Furthermore, resulting from the use of nonspecific immunosuppressive agents the characteristics of infectious disease and its therapeutic drugs summarized.

The summary mainly outlines the studies acquired by the author, in addition in order to succeed in organ transplantation, not only operative technique as a surgeon, but also extreme amount of basic knowledge are required. Without these processes, newly developed methodology in immunosuppression would not have been generated without acquiring these knowledge and expertise. And let's make is viable "Fabricating transplantable organs from own cells" which is the author's lifework.

Mechanisms of immunological rejection

The main reason for immunological rejection is activation of T cell of the recipient against transplantation angigen, which is called specific reaction (antigen recognition) to be followed by a nonspecific reaction (In English it is called non-immunological; inflamation is a port of immunoreaction). The latter reaction is divided into two symptoms; one is the end stage of acute rejection and the other is extreme chronical rejection gradually progressing in a long term.

When a T cell responds to transplantation antigen, there exist two types of recognitions; one is that transplantation antigen is directly recognized as alloantigen coping with T cell (direct recognition) and the other is that alloantigen is processed with macrophages once and emerges on the major histocompatibility complex (MHC) of the receipient and then T cell of the recipient recongizes it (indirect recognition) (Figure 1). In both cases, the recognition of transplantation antigen is conveyed through T cell receptor to the promoter of IL-2 gene in the recipient's intranuclear T cell. Plenty of molecules let antigen presenting cell (APC) and T cell adhere each other besides T cell recepter emits sub-signal together with main signal simultaneously (Figure 2).

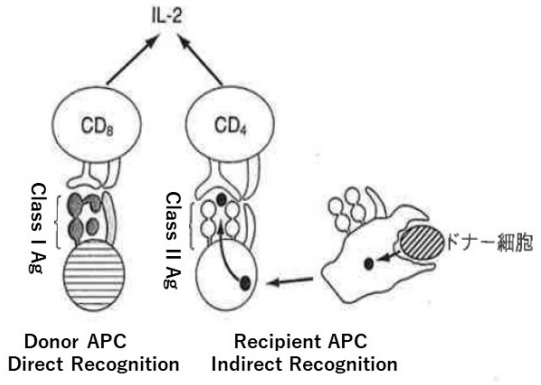

Figure 1: Interaction between Class I or II Ag to T Cell Subsets

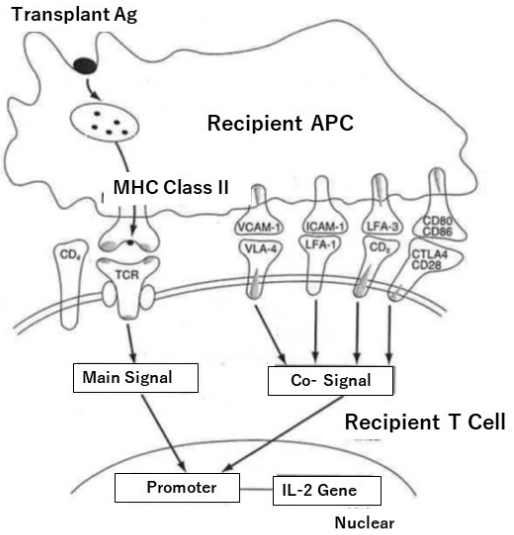

Figure 2: Cell Adhesion Molecules and T cell activation

The author had been studing mechanism of rejection of major histocompatibility complex (MHC) class I and/or II-disparate grafts using murine skin grafting (1-7) (Figure 3). Histological and immunohistochemical studies of the rejection phenomenon showed that only CD8+ cells infiltrated at the site of the epithelial tissue of MHC class I-disparate graft (2). (Figure 3-C).

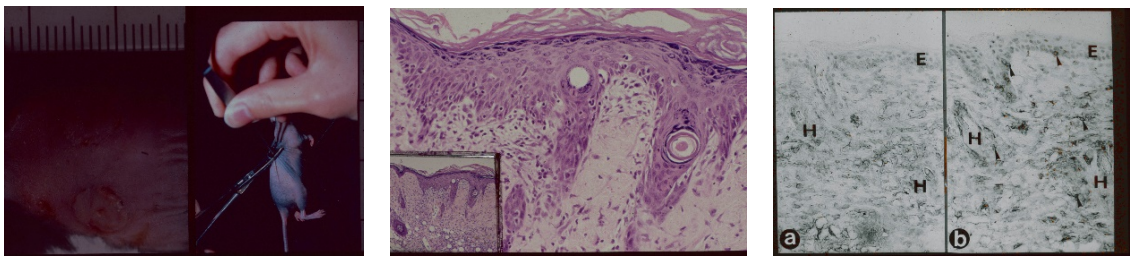

Figure 3 A B C

Perfect survival of MHC class I-disparate grafts were attained in thymectomized recipients treated

with anti-CD8+monoclonal antibody (3). On the other hand, MHC class II-disparate grafts were mainly rejected by CD4+ cells (6). These phenomenon have been considered to be reasonable from the points of interaction of MHC restriction with CD4 and CD8 T cell subset.

However, we found generation of MHC class II-reactive CD8+ cells in mice after class II-disparate skin graft rejection (5) (Figure 4). It was worthwhile saying that these long-surviving allo-class I grafts were rejected in the absence of CD8+ cells by stimulation with allo-MHC class I + II-disparate graft as the second stimulation(7). Iimmunohistochemical studies revealed that under that condition, a large number of CD4+ cells infiltrated into the epithelial tissue of these long-surviving class I grafts, which were going to be rejected 2-5 d after the transplantation of a second graft with MHC class I + II difference. This result directly shows that CD4+ cells are able to became effectors for the rejection of allo-MHC class I skin graft (Figure 5)

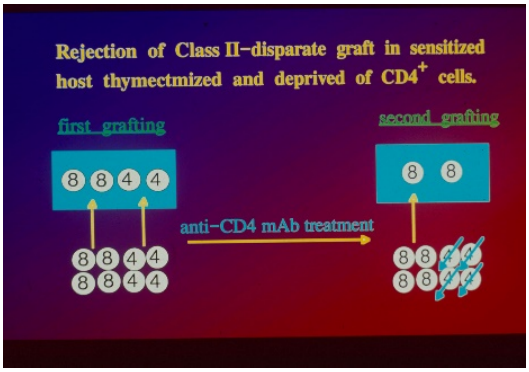

Figure 4

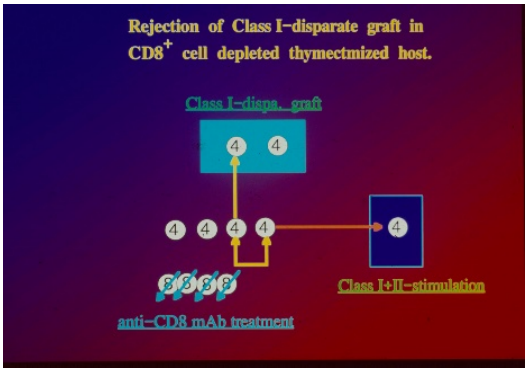

Figure 5

Immunoreaction advances as a chain reaction after the first recognition of recipient T cell as a transplantation antigen. This transplantation antigen recognition is a phenomenon acting as a base of tolerance induction experiments, which lead rapidly either to a reaction (immune tolerance) or harmful reaction (rejection).

As for immunological tolerance status, supreme concept has been disclosed from the early stage of 1990s when the author had started transplantation immunology. Therty years past now, I think that nothing superior to the concept of that time has been achieved up to now but more detailed mechanism has been elucidated so far. Because T cell clone reacting modes as a classic concept are ①Clonal anergy (Reacting T cell adhered to transplantation antigen gets paralyzed) and ②Clonal delation (Reacting T cell disappears due to apotosis; death of cells). On the other hand, as for the helper T cell (Th) there exist two different outcomes; ①Th1 which generates IL-2 and INF-γ is induced or Th2 which generates IL-4 and IL-10 sometimes enlarges the width of clone size. The former Th1 induces acute immunosuppressive reaction and the latter Th2 elicits humoral immunity and induces immune tolerance while causing immunosuppresive state. It used to be called inhabitor as well as regulator with the meaning of adjusting. FoxP has been discovered as a gene of this regulator T cell and

consequently the existence of regulatory T cell has been proven (8). In basic immunology the methodology to induce donor-specific immune tolerance is developed by inducing immunoreaction. However, the immunosuppresive agents used for clinical practices are to stop these recognizable reaction of transplantation antigen and turn out to be in nonspecific immunocompromised state.

Molecular mechanism of immunosuppressive agents

Immunosuppresive agents currently used are to surpress the emergence of IL-2. These drugs are segmented as below by the points of application (Figure 6).

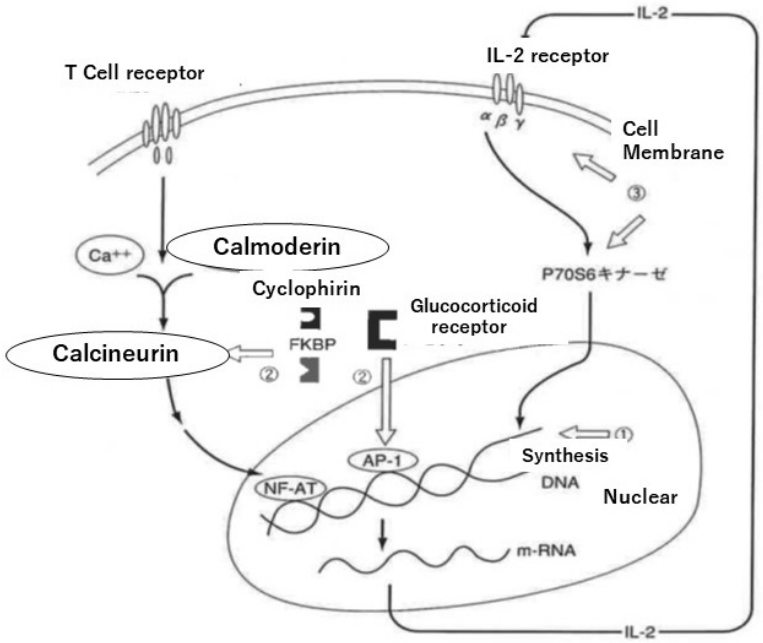

Figure 6: Molecular Mechanism of Immunosuppressive Drugs

1. In case of impeding nucleic acid composition

Azathioprine, mizoribine, mycophenolic acid, RS61443 are classified in this category. Basically they have characteristics similar to anticancer agents and also they act as suppressor for cell proliferation and in the consequence they gather attention because they are used as adjuvant and preventive agents from chronicity rejection in case of ABO incompatible transplantation.

2. In case of suppressing intracellular signal transduction in time for IL-2 generation

Cyclosporine and tacrolimus are in this segment as drugs to suppress the calcineurin effect (Calcineurin blocker) and work as transcription factor in the cytoplasm which induces existing IL-2 gene in the nucleus of the lymphocyte After cyclosporine and tacrolimus are combined with iminofirin (protein in the cell) called cyclophilin and FKBP respectively and then they act on calcineurin. Steroid is also categorized here because it suppresses the emergence of m-RNA in IL-2 after combining with intracellular receptor (heat shock protein 90). Currently these are basic drugs for

immunocuppression. The most important thing is the efficacy of these drugs how they inflow and outflow from lymphosite concerning degree and duration of efficacy.

3. In case of jeorpadizing information from IL-2 receptor

Rapamycin and deoxyspergualin are classified here and are combined with FKBP and heat shock protein 70 respectively. Each one has characteristics different from calcineurin inhibitor and especially rapamycin provides with cirrhosis progress prevention and has been paid attention in case of liver transplant for the C type cirrhosis in clinical practices.

As for organ transplant clinics, mixture of these drugs with different points of application based on calcineurin inhibtor are used. This is because in general experiments for the combination effect (side effect) become hard to be performed once clinical practices (Phase IV) start resulting in the fact that fundamental experiments slow down accordingly. In addition, there have been few reports based on the EBM (evidence-based medicine) in Japan and the researches for basic studies and clinical application in regard to such combinations should be positively developed in the future (9). Furthermore, these immunosuppressants differ greatly in the degree of rejection in accordance with types of transplanted organs. It is necessary to create various kinds of methods and tips how to deal with these rejection variations respectively.

4. Others (such as receptor antibodies)

Unlike immunosuppressant extracted from the above-mentioned mold, there is another targeting substances to be emerged in living bodies which cause immunoreaction. In old days there existed OKT-3 which targeted the whole lymphocyte, but nowadays IL-2 receptor antibody is mainly used in clinical practices in foreign countries. Immediate permission to use those drugs is expected in Japan, but a delay in developing clinical trials in Japan has been affecting seriously as well as that of brain death transplant. On the other hand, it is still experimental but there exist a substance which shows immunoreactive strength among proteins generated in living body. These protein substances are completely different from the above stated 1, 2 and 3 which are drugs created *ex vivo*, they are creations by the gene which Homo sapiens are originally endowed.

Therefore, they are less toxic and physiological. So-called gene drug identifying types of genes used as medicine gathers utmost attention as genome innovative drug development. A gene patent "war" with North American and European countries has been picked up as a big topic. But the author deeply considered that it was more important to organize a team to study on the application systematically as one group instead of confronting with researchers in foreign countires indivisually. Twenty years ago, the author also have been studing chronopamacological approach of these immunosuppressive drugs (10-22). While chronophalimacological effect is small, we should study

more how to use these immunosuppressive drugs.

Infectious disease

The clinical application of immunosuppressant has brought an innovation on graft survival ratio of transplanted organ, but it also has broght a danger to fight always against infectious disease after transplant. In late years, close to 10% of causes of death have directly come from infectious diseases in living donor liver transplant which has been based on long-year experiences and expetise in Japan. Particularly, attention should be paid on the fact that these fatal infectious diseases have ocurred in the early stage of post-operation (less than one month).

The infectious disease is classified generally into infections of bacteria, virus and fungus shows infectious disease caused by the immunocompetent cell disorger (Figure 7).

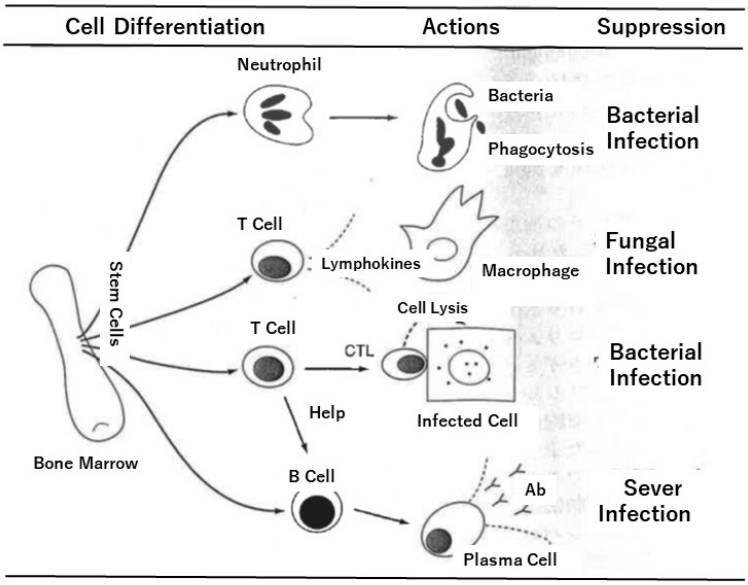

Figure 7: Immunocompetent Cells and their actions

As referred earlier, currently- used immunosuppressant is nonspecific, it influences on most of immunocompetent cells. Infectious disease is characterized by types of transplanted organs, therefore, it should be considered by merging all the above facts together. Furthermore, it is affected by the conditions of antimicrobial use at transplant facilities, and the onset such as the MRSA occurs after transplantation (it is often the case that inapparent infection has appeared apparent).

More than half of infectious diseases are occupies by bacterial ones even narrowing down to those of live donor liver transplants. Furthermore 80% of them have occured within two months of postoperative periods. Pathogenic bacterias derive from both aerobic gram positive and negative. In case of treatment, it is important to limit the usage of antimicrobial agents for a short period in

consideration of phlogogenic fungus sensitivity.

Among viral diseases, cytomegalovirus (CMV) infectious disease is important and it is often seen in 1-2 months postoperative period. There are many cases that diagnosis is difficult only from clinical manifestations, but in recent years genetic screening for the virus has become viable for the purpose of treatments including other virus infectious diseases.

More than 80% of fugnal infuctious disease is dominated by genital candidiasis symptom. Among all types of mycrosis. To identify the cause from this infectious disease in deceased patients is extremely high, but the symptom shows up apparently on the patient body similar to seriously ill patient from other diseases.

It is important to start treatment in the early stage against these fungus infection due to the fact that it mainly comes from T cell disorder. Currently, as anti-viral drug and antifungus act as a key to the treatment. Particularly, highest attention should be paid to side effects (drug toxic reaction). In addition, it is extremely important to pay attention to "Make allowances to prescription drugs" taking into consideration that infectious disease after transplant has been mainly caused by immunosuppressant agents. In a sense, pharmaceutical knowledge is extremely important to understand immunity of organ transplantation, and "try & error" ratio increases if experiences of person only come from clinical knowledge. It is applicable for all clinical practices that experiences based on knoledge is necessary to save suffering children to maximize tips to “Make allowances” based on delicate team approched medicine.

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