Genetic modulation of immature T lymphocytes and its application

Yousuke Takahama

Division of Experimental Immunology, Institute for Genome Research, University of Tokushima, Japan

Abstract: T lymphocytes are the cells that play an essential role in regulating immune responses. The thymus is the organ in which T lymphocytes are generated. Our laboratory has investigated molecular signals that determine cell fate during T lymphocyte development in the thymus. To this goal, we have devised a technique in which one may efficiently introduce foreign genes into immature T lymphocytes. The somatic gene-transfer into developing T lymphocytes are likely useful to restore various immunodeficiencies and to establish immune tolerance to any introduced genes. The genetically engineered immune tolerance may be applied to reduce allergies and autoimmune diseases, as well as to sustain gene therapies by allowing prolonged survival of therapeutic vectors. J. Med. Invest. 48 : 25-30, 2001

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INTRODUCTION

Most T lymphocytes develop in the thymus. The thymus is a small, isolated organ that can be manipulated *in vivo* and can be analyzed *in vitro*. Developmental biology of T lymphocytes investigates how the hematopoietic precursor cells are induced to develop to mature T lymphocytes in the thymus. Specifically, interesting questions to be asked include issues such as (i) how immature T lymphocytes are selected for life and death according to their antigen-recognition specificity, (ii) how multipotent precursor cells are committed to become individual T lymphocyte lineages, and (iii) how developing T lymphocytes relocate along the differentiation into, within and out of the thymus.

In the thymus, immature lymphoid precursor cells begin to rearrange the V (D) J segments of T-cell antigen-receptor (TCR) genes. The V (D) J rearrangement is an irreversible alteration of genomic DNA in the nucleus, so that individual lymphocytes cannot predict the antigen-recognition specificity before they express TCR chains on the cell surface (1, 2). Consequently, immature T lymphocytes in the thymus, also called thymocytes, may express various kinds of antigen-recognition specificity, including harmful specificity, useful specificity, and useless specificity. The developmental fate of immature thymocytes is determined by the interaction between the TCR that they express and its ligand, peptide-MHC complex, expressed in the thymus (3, 4). High avidity TCR interactions cause apoptosis of thymocytes, deleting harmful self-reactive cells, a process referred to as negative selection. Thymocytes expressing TCR that fail to interact with MHC ligands are also destined to die within the thymus, eliminating useless T cells that are unable to recognize foreign peptide-loaded self-MHC molecules. Low avidity TCR interactions, on the other hand, elicit the signal that allows immature thymocytes to differentiate into mature T lymphocytes, a developmental process referred to as positive selection. Thus, positive selection of thymocytes enriches useful T cell clones that can recognize foreign peptides presented by self-MHC molecules. It has been shown that cell surface events such as avidity and valency of TCR ligation by peptide-MHC com-

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Address correspondence and reprint requests to Yousuke Takahama, Division of Experimental Immunology, Institute for Genome Research, University of Tokushima, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-9453.

plex are involved in determining the opposite destinations (life and death) of immature thymocytes (5-8). However, it is still largely unknown how these cell-surface events are transmitted within thymocytes to determine life-death destinations of the cells.

Majority of positively selected thymocytes are induced to develop into either one of CD4+CD8- T cells (mostly helper T cells) or CD4-CD8+ T cells (mostly killer T cells). Even though TCR signal intensity as well as other signals such as Notch have been shown to be involved in lineage choice between CD4+CD8- T cells and CD4-CD8+ T cells (9-11), it is largely unclear how the choice between the T cell lineages is made. It is also unclear how multipotent precursor cells that have migrated to the thymus are destined to become T lymphocytes rather than other cell lineages such as B lymphocytes and other hematopoietic cells.

Developing T lymphocytes relocate through the thymus during differentiation. Immature lymphoid precursor cells immigrate into the thymus, developing thymocytes relocate within the thymus from the cortex to the medulla, and finally mature T lymphocytes emigrate from the thymus (12-14). How the cellular movement along T lymphocyte development is controlled is largely unknown.

Exploring these issues of T cell development would be relevant for better understanding of complex biological systems specialized in multi-cellular organisms, as well as for offering better treatment of various clinical situations in which immune systems are involved. To examine these issues and to aid clinical applications, our laboratory has devised a somatic cell-gene transfer technique in which one can express a given gene in developing T lymphocytes.

GENE-TRANSFER INTO DEVELOPING T LYMPHOCYTES

To genetically modulate developing T lymphocytes, the manipulation of embryonic cells such as transgenesis and ES-mediated homologous recombination has been most widely used (15-18). These techniques have greatly contributed to the current understanding of molecular mechanism for T lymphocyte development. However, genetic manipulation of embryonic cells alone cannot directly allow *in situ* analysis of the cellular development within the thymus organ.

On the other hand, the analysis of T cell development in organ culture of mouse fetal thymus lobes was first established in the early seventies (19-21). The fetal thymus organ culture (FTOC) technique serves a unique *in vitro* cell culture system in that functional T cells are differentiated from immature progenitor cells (22). T cell development in FTOC very well represents T cell development during fetal life, even representing the time course. FTOC allows *in vitro* T cell development isolated from any further cellular or humoral supplies by other organs ; thus, it is suitable for the addition of any reagents, such as drugs and antibodies to the culture, for examining their effects on T cell development. FTOC is also useful for the analysis of T-lymphopoietic capability by hematopoietic progenitor cells.

Recently, retroviral gene transfer has been successfully used for a wide variety of cells including hematopoietic cells (23-28) and developing B lymphocytes (29, 30). Retrovirus-mediated gene transfer has several advantages over the transgenic techniques, including rapid and close analysis of specific cellular events in vitro and potential application for gene therapy. However, retrovirus-mediated gene transfer often suffers from technical difficulties such as low efficiency, hampering applications in various cell types. Consequently, attempts to introduce exogenous genes using retroviruses have gained limited success on developing T lymphocytes (31-37).

During the last three years, a successful and reproducible gene-transfer technique for developing T lymphocytes has been established in our laboratory (22, 38, 39). The short-term co-culture of immature thymocytes in suspension with high-titer retrovirus-producing cells in the presence of interleukin-7, a cytokine that maintain the survival of immature lymphocytes, seems to be the key for highly efficient gene transduction (Fig. 1). We have constructed recombinant retroviruses expressing green fluorescence protein (GFP) along with a protein of interest, using the internal ribosomal entry site (IRES) sequence (Fig. 1). The co-expression of GFP is useful, as gene-transferred cells could be readily detected and sorted using flow cytometry. Immature thymocytes have been successfully infected with these retroviruses in a short-term suspension culture in the presence of interleukin-7, and were examined for their developmental capability by transferring to the thymus organ culture. The fate of isolated GFP-expressing cells can be traced under the fluorescence microscope or by multi-color flow cytometric analysis, so that the developmental potential of genetically manipulated cells may be directly evaluated in FTOC (Fig. 1).

Using the retroviral gene-transfer method, we have shown that ERK kinase and p38 kinase characterize TCR signals that mediate positive and negative

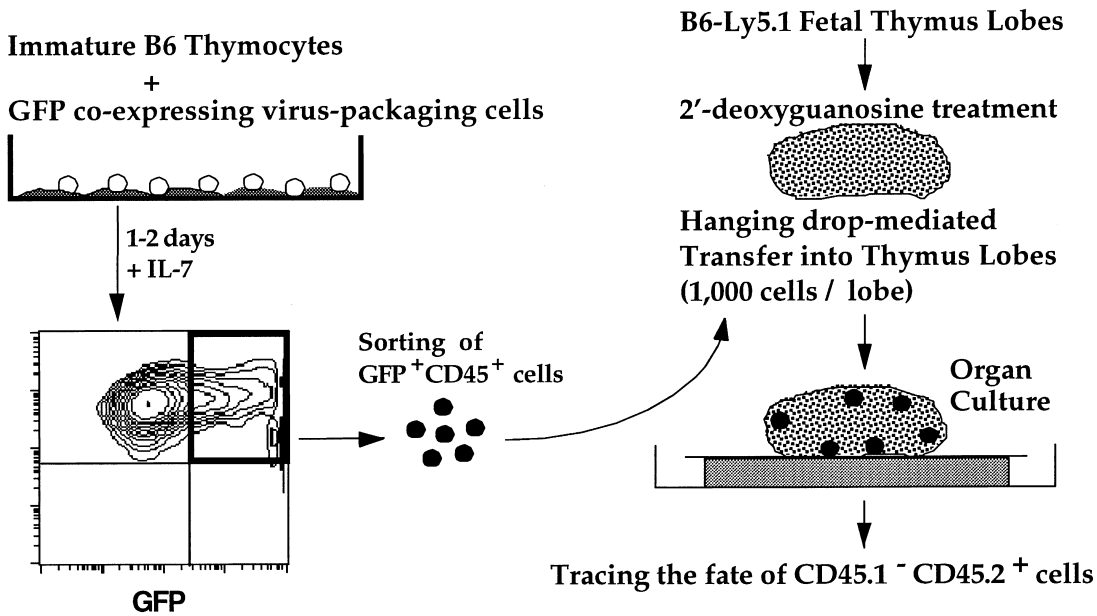

Fig.1. Retroviral infection of developing thymocytes.

selection of developing thymocytes, respectively (40). Quantitative differences in TCR ligation at cell surface have been shown to determine the fate of immature thymocytes during positive and negative selection (5-8). Our results showing differential involvement of ERK and p38-kinase signaling cascade in positive and negative selection suggested that differential TCR ligation in developing thymocytes may activate differential MAP kinase pathways, leading to opposite destinations of immature thymocytes. We have also shown that Pref-1 expressed on the surface of thymic epithelial cells increase the expression of HES-1 transcription factor in developing thymocytes, thereby maintaining the survival of thymocytes (41). Thus, the retroviral technique that we devised is an efficient and reliable method for gene-transfer into developing T lymphocytes.

POTENTIAL APPLICATION OF GENE-TRANSFER IN IMMUNODEFICIENCIES

Gene-transfer for immature T lymphocytes is likely useful for genetic manipulation of T lymphocyte development. The most straightforward application would attempt to restore of immunodeficiencies by the transfer of genes that promote the development of T lymphocytes (Fig. 2). For example, immunodeficiency in ZAP-70 deficient patients could be best restored by transferring the ZAP-70 gene into immature thymocytes (42, 43). Such a restoration could be applied not only for congenital immunodeficiencies such as X-linked severe combined immune deficiency and selective T cell deficiency, but also for acquired immunodeficiencies such as AIDS and immune suppression by chemotherapy or after transplantation.

POTENTIAL APPLICATION OF GENE-TRANSFER IN GENE THERAPIES

The gene-transfer for immature T lymphocytes is also viewed as a very efficient vehicle to introduce gene products into the thymus and developing thymocytes. It is therefore conceivable that the gene-transfer into immature T lymphocytes could efficiently induce immune tolerance to the products of any foreign genes. The gene-specific induction of immune tolerance offers at least two types of clinical applications. First is the treatment of allergy, in which the immune system reacts to foreign molecules that are not expected to be immunogenic. The introduction of the allergen gene to immature thymocytes may cause tolerance to the allergen, so that allergic reactions would be expected to diminish after the gene-transfer. This line of genetic modulation of immune responses can also be applied to reduce auto-reactivity in various autoimmune diseases. Second is immunological support for gene therapies of any cell types (Fig. 3). It has been well appreciated that one of the most serious problems ingene therapy is the rejection of vector-introduced cells by exerting immune responses to vector-derived gene products (44-47). Such immune responses dramatically preclude the prolonged effectiveness of introduced genes. In addition, once the immune response to the introduced vector is established, immune cells would quite efficiently attack the cells that are newly introduced with the vector.

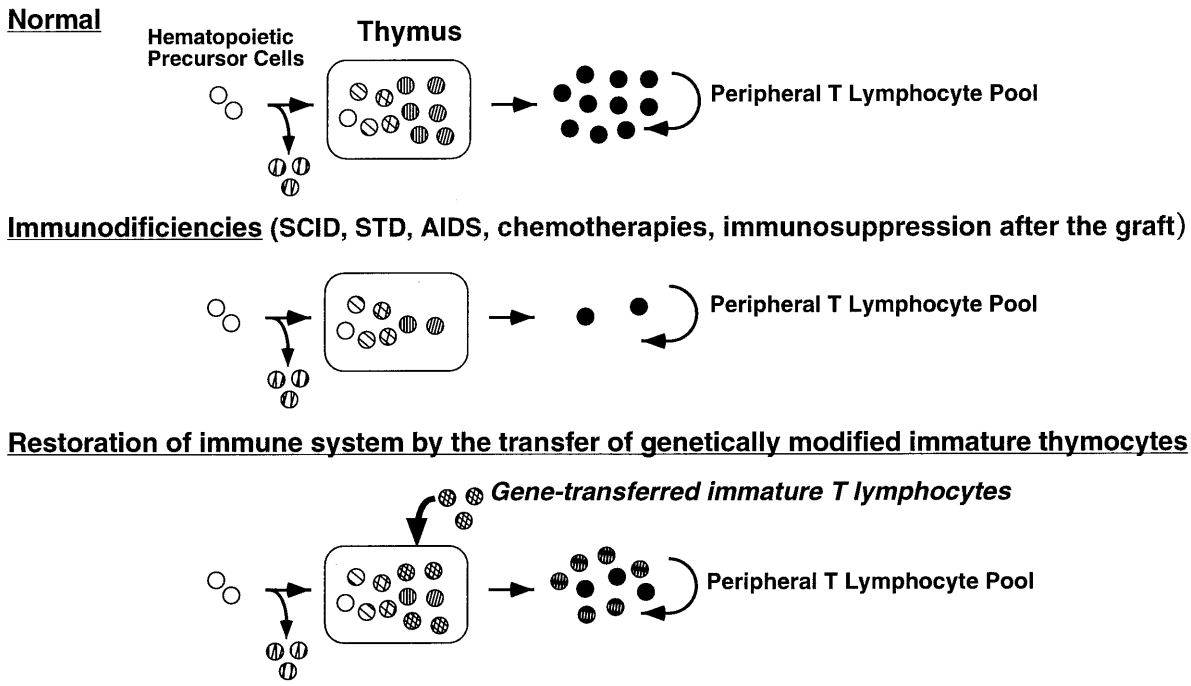

Fig.2. Strategies for gene therapies of immune deficiencies.

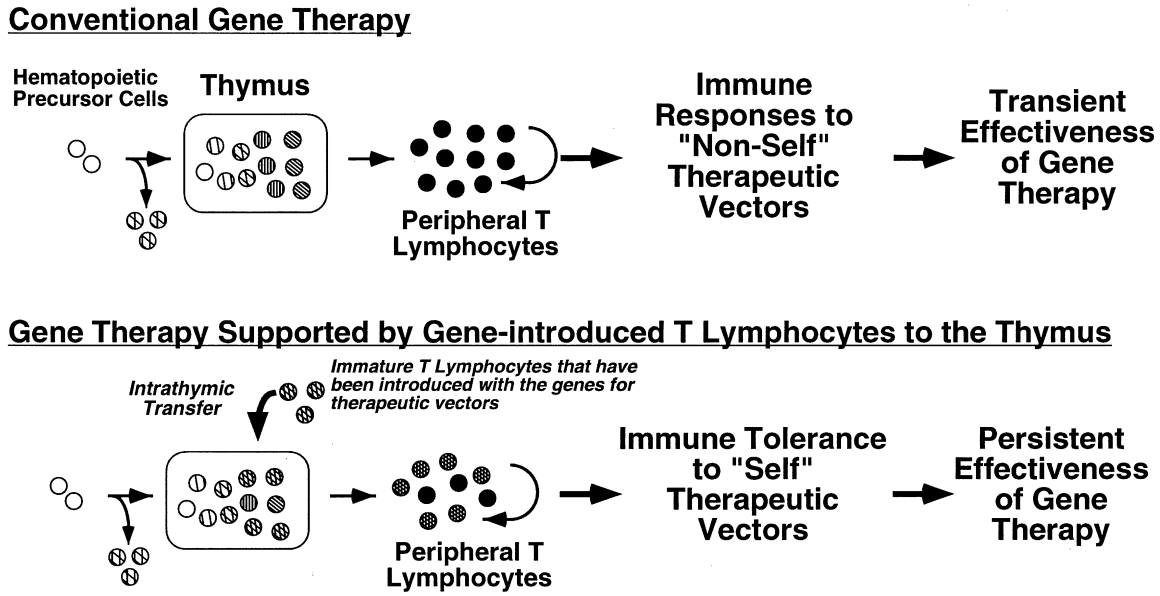

Fig.3. Gene therapy supported by gene-introduced T lymphocytes to the thymus.

It would thus be nearly impossible to expect efficient therapeutic effects by repeated treatment with the same vector during gene therapy.

On the other hand, immune responses are crucially regulated by T lymphocytes. T lymphocytes are the cells that immunologically distinguish self to be protected from non-self to be attacked. It is therefore conceivable that one can specifically repress immune responses to a foreign molecule by displaying that molecule in the thymus as a 'self' molecule. The vector transfer of the therapeutic gene into the thymus, for example by the intrathymic administration of immature thymocytes that have been gene-transferred with the same vector, would induce immune tolerance to the vector (48). Consequently, subsequent gene therapy is expected to be sustained without being disrupted by immune reactions (Fig. 3).

These applications of gene-transfer are currently being evaluated in our laboratory. We believe that the evaluation and further improvement of genetic

modulation of the immune system offers a promie of overcoming immune diseases in the new century.

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