The study of regulatory T cells and NKT cells in Japan: a historical perspective

**Hisashi Arase**¹² and **Ken-ichiro Seino**³⁴

¹Laboratory of Immunochemistry, World Premier International Immunology Frontier Research Center and ²Department of Immunochemistry, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0081, Japan
³Division of Bioregulation Research, Institute of Medical Science, St Marianna University Graduate School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki-City, Kanagawa 216-8511, Japan
⁴Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan

**Keywords**: NKT cells, regulatory T cells, suppressor T cells

**Abstract**

Immune regulation plays an important role in maintaining homeostasis of the immune system. A number of Japanese immunologists have made significant contributions to the elucidation of the mechanisms of immune regulation. In particular, lymphocyte populations that could regulate immune responses—for example regulatory T cells and NKT cells—have been extensively analyzed. Here, we present an overview of research on immune regulation by highlighting the work of several Japanese contributors.

**Introduction**

The immune system plays a pivotal role in host defense; however, excessive responses can be harmful to the host. Immune cells can respond to various non-self and self-antigens; however, self-activity is normally deleted or depressed by the immune system itself. Mechanisms of immune regulation are therefore considered to be essential to maintain homeostasis of the immune system.

A central question facing immunologists at the moment is precisely how the immune system is positively and negatively regulated. Only a harmful immune response must be inhibited and, so far, various mechanisms that are involved in this negative regulation have been elucidated extensively; for example clonal deletion, anergy of self-reactive cells, inhibitory signals by cell surface receptors or production of inhibitory cytokines by immune cells have been considered to play an important role in negative immune regulation. An additional mechanism—mediated by antigen-specific regulatory T (Treg) cells—has been suggested for a long time.

In order to understand the historical contribution of Japanese immunologists to research into Treg cells, in our article, we begin by discussing suppressor T cells. We then discuss the emergence of Treg cells. Also in this issue of the journal, Shimon Sakaguchi provides an intriguing and comprehensive review on Treg cells. Japanese immunologists have also contributed a lot to the understanding of the complexity of other T cell subsets. As well as Treg cells, it is now known that there are several T cell sub-populations such as T\(_{h1}\), T\(_{h2}\), T\(_{h17}\), CTL, γδ\(^+\) T and NKT cells and that these T cell subsets play an important role in immune response. We describe the contribution of Japanese scientists to the discovery of NKT cells in particular.

**The concept of suppressor T cells**

In the late 1960s, it was discovered that the immune system consists of two major lymphocyte types—B cells that are responsible for antibody production and T cells that are involved in delayed-type hypersensitivity and antibody responses by co-operation with B cells. In early 1970s, Tomio Tada Chiba University and, later, Tokyo University, (Japan) and Richard K. Gershon (Yale University, New Haven, USA) provided the important concept that T cells not only had these helper functions for the immune response but also could depress immune responses (1, 2). They showed that adoptive transfer of T cells from mice that had been immunized with a large dose of antigen suppresses antigen-specific antibody production in recipients.

After their pioneering work, the accumulation of many experiments over the next few years demonstrated the activity of suppressor T cells in the regulation of immune response.
response to several antigens, such as sheep red blood cells, proteins, haptns, synthetic polypeptides and tumor cells. Suppressor T cells were found to be antigen specific and their physicochemical nature was extensively studied. Consequently, several models were proposed for suppressor mechanisms; however, the final answer remained unsolved, owing to a limitation of molecular technology at that time.

On the other hand, clonal deletion and anergy of self-reactive T cells were demonstrated by using specific anti-TCR mAb and TCR-transgenic mice in the late 1980s, and self-tolerance mediated by these mechanisms was extensively analyzed. It should be noted that enormous study of suppressor T cells contributed a lot to the subsequent analyses of immune regulation.

The emergence of Treg cells

Because of the absence of definitive molecular evidence of suppressor T cells, as described above, interest among immunologists rapidly waned after the 1980s; however, because of the important immunological concept raised by the analyses of suppressor T cells, another important stream of research on immune regulation was born in Japan; this research later became extremely important and led to the concept of Treg cells. Yasuaki Nishizuka and Teruyo Sakakura in the Aichi Cancer Research Institute (Nagoya, Japan) found that neonatal thymectomy 2–4 days after birth can induce various organ-specific autoimmune diseases including oophoritis, gastritis, and thyroiditis (3).

When he was in Nishizuka’s laboratory, Shimon Sakaguchi studied cellular events in autoimmune diseases that occur after thymectomy. They found that inoculation of normal T cells, in particular thymocytes (probably CD4+ or CD4-CD8-), from untreated syngeneic animals inhibited the development of autoimmunity (4). These initial findings indicated that normal animals harbor not only potentially pathogenic self-reactive T cells but also T cells (probably CD4+) that suppress autoimmunity.

Sakaguchi et al. (5) continued to investigate this phenomenon and looked for the sub-population responsible for the suppression of autoimmunity. They showed that the suppressive T cell sub-population exists in the mouse CD4+CD5high population, and other research groups indicated that similar suppressive cells are found among rat CD4+CD45RClow T cells (6) or in rat RT6.1+ T cells (7). Sakaguchi further narrowed down the sub-population responsible for suppression and finally found that CD25 is a specific candidate marker for the population (8); CD25 (IL-2Ra) was previously well known as an activation marker for T cells. The thymus-derived CD25+CD4+ T cells, confined in the CD5high and CD45RBlow fraction of CD4+ T cells, then came to be called ‘naturally occurring Treg cells’ and were shown to have suppressive potential in various immune responses—not only autoimmunity but also transplant tolerance or tumor immunity.

Sakaguchi greatly contributed to the elucidation of Treg cell biology, especially at a molecular level. His group found that some cell surface molecules, such as glucocorticoid-induced tumor necrosis factor receptor family-related receptor (also known as tumor necrosis factor receptor superfamily, member 18), cytotoxic T-lymphocyte-associated protein 4 and folate receptor 4, are critical for maintaining the function of Treg cells and are also good markers for them [reviewed in (9)]. His group identified that a transcription factor, forkhead box p3 (Foxp3), is a critical regulator of Treg cell development and function (10).

Recently, it has been reported that Foxp3-expressing T cells can be generated in vitro by culturing naive T cells with transforming growth factor β (TGF-β). When IL-6 is added to the culture, the T cells became a different T cell subset—Treg cells. In the context of Treg cell differentiation, the study of TGF-β-induced Treg cells opens a new avenue for study (11).

Because of their substantial potential for regulating the immune system, Treg cells are being investigated thoroughly by many immunologists worldwide, and this T cell subset is expected to be used in some clinical settings in the near future. The details of the biology and function of Treg cells are reviewed in the article by Sakaguchi in this issue.

The discovery of NKT cells and their role in immune regulation

Although CD4+ and CD8+ T cell subsets conventionally express a TCRγδ heterodimer with a broad range of V, D and J sequences in the α and β chains, a novel T cell population that has a skewed (i.e. relatively invariant) expression in its TCRβ repertoire was reported by Fowlkes and by Macdonald (12, 13). Independently, Masaru Taniguchi and colleagues (14, 15) at Chiba University cloned and identified an ‘invariant’ Vα14–Jα281-containing TCR chain from a suppressor T cell hybridoma. Analyses of Vα14–Jα281-expressing T cells suggested that the cells expressing this ‘invariant’ TCR proliferate at a high frequency and apparently seem to belong an autoreactive repertoire different from conventional T cells. A similar T cell population was also detected in liver (16).

In addition, a T cell sub-population that expresses a skewed TCRγδ repertoire and expresses an NK cell marker, NK1.1, was identified in CD4+ T cells (17). Because one unique characteristic of T cells with the skewed TCR repertoires was expression of an NK cell marker, this population was later called ‘NKT cells’. These cells produce large amounts of IL-4 and IFN-γ (18), and differentiation of NKT cells is restricted by CD1d (a non-classical MHC molecule), unlike conventional T cell populations (19, 20). NKT cells were therefore thought to play a unique role in immune regulation. Interestingly, the Vα14 TCR has been revealed to be a representative TCR for murine NKT cells (21). These findings suggested that suppressor function observed by certain T cell subsets might be partially mediated by NKT cells. Direct correlation between suppressor T cells and NKT cells still remains unclear, however.

In 1997, there were great advances in the study of NKT cells. Masaru Taniguchi and colleagues (22) found that development of NKT cells is abolished in mice lacking Jα281. Their findings indicated that specific combination of Vα14 and Jα281 in the TCR is required for the development of NKT cells. This suggested that specific antigen recognition is required for NKT cell development. Another finding
by Taniguchi and his colleagues is the identification of putative ligand for NKT cells. They have found that α-galactosylceramide (derived from a marine sponge) is specifically recognized by NKT cells when presented on CD1d (23). More interestingly, activation of NKT cells by α-galactosylceramide induced tumor rejection. These findings provided a possible application of NKT cell ligands for tumor therapy.

After the publication of these papers, a lot of researchers around the world—including Japanese researchers—started to use these experimental reagents and their findings greatly contributed to elucidate the functional roles of NKT cells. For example, the roles of NKT cells in anti-tumor responses, infection by bacteria or fungi and maintenance of allograft tolerance were elucidated (24). Especially, Yamamura et al. at the National Center of Neurology and Psychiatry in Tokyo found a novel derivative of α-galactosylceramide, OCH, that has a potential to induce T\(_{H}2\) response and inhibits experimental autoimmune encephalomyelitis (25). In this way, the novel function of NKT cells in the immune system, either up-regulating or down-regulating responses, is expected to be useful for clinical application—not only for cancer immunotherapy but also in the treatment of autoimmune diseases (26).

Conclusions

As described in this perspective, a lot of Japanese immunologists have contributed to the understanding of regulatory mechanisms of immune system. Further elucidation of the cellular and molecular processes underlying the immune-regulatory responses will help to establish new strategies for the treatment and prevention of immunological diseases and for the control of a wide spectrum of physiological immune responses.

Acknowledgements

We would like to thank Drs. Masaru Taniguchi, Masato Ogata and Hitoshi Kikutani for critical reading of the manuscript.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxp3</td>
<td>forkhead box p3</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor β</td>
</tr>
<tr>
<td>Treg</td>
<td>regulatory T</td>
</tr>
</tbody>
</table>

References


Study of regulatory T cells and NKT cells in Japan