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Review Article

T cells and autoimmune diseases

Kazuhiko Yamamoto

Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Abstract

The roles of antigen-specific immune reactions are discussed in different types of autoimmune diseases. Antigen-specific reactions of T cells are carried out at the level of clones. In this regard, we have established a novel method for analyzing T cell clonality. Analyses of several autoimmune disorders suggest the importance of antigen-specific T cell clones. Furthermore, our findings cast doubt on the idea of epitope spreading, which has been proposed recently for T cell activation in autoimmune processes. Our system also appears to work as a new method for the identification of target antigens recognized by such accumulated T cell clones.

Key words: autoimmune disease, T cell clone, T cell receptor.

Antigen-specific immune reactions in different types of autoimmune diseases

There is a spectrum of autoimmune diseases. At one end of this spectrum are organ-specific autoimmune diseases, such as Hashimoto's disease, which is characterized by autoantibodies specific to thyroid antigens as well as mononuclear cell infiltrations of the thyroid gland. At the other end of the spectrum are systemic autoimmune diseases. Systemic lupus erythematosus (SLE) is a representative example and the lesions and autoantibodies are not confined to any one organ. Between these two extremes, there are intermediate conditions in which autoantibodies are organ non-specific but the lesion is localized to a certain organ.

Correspondence: K Yamamoto, Department of Allergology and Rheumatology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: yamamoto-tky@umin.ac.jp

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In organ-specific autoimmune diseases, antigens in the target organ appear to drive the autommune response.[1] Animal experiments, such as immunizing animals with an organ-specific antigen with a strong adjuvant, clearly show that a specific immune response to the self-antigen can elicit autoimmune diseases. Evidence that the susceptibility to certain autoimmune diseases is controlled by the major histocompatibility complex (MHC) also suggests that T cells are deeply involved in this process.[2]

In contrast, in systemic autoimmune diseases, several antigen-non-specific functional disturbances have been reported in various immune cells. They are, for example, activation of polyclonal B cells, abnormal distribution of T cell subsets, altered cytokine production, aberrant signals for apoptosis and functional alterations of monocytes and macrophages. In contrast, there is a lack of knowledge about antigen-specific immune responses. Therefore, several investigators believe that antigen-specific immune responses do not play important roles in systemic types of autoimmune disease.[3]

T cells in antigen-specific autoimmune diseases

T cells recognize an antigen by means of T cell receptors (TCR). Most T cells express TCR consisting of α- and β-subunits, while others express γ- and δ-subunits. In each T cell, the TCR gene has acquired vast variability by means of gene rearrangement and random deletion and insertion of nucleotide sequences on the borders of the V, D and J regions. When a population of T cells with this infinite variability of TCR reacts to an antigen, the reaction activates T cells that have TCR recognizing the complex of the MHC molecule and the antigen peptide. This is because the reacting TCR transmit growth signals in the cells and these cells proliferate and grow. The population of T cells having the same rearranged TCR are referred to as a clone. Therefore, antigen-specific

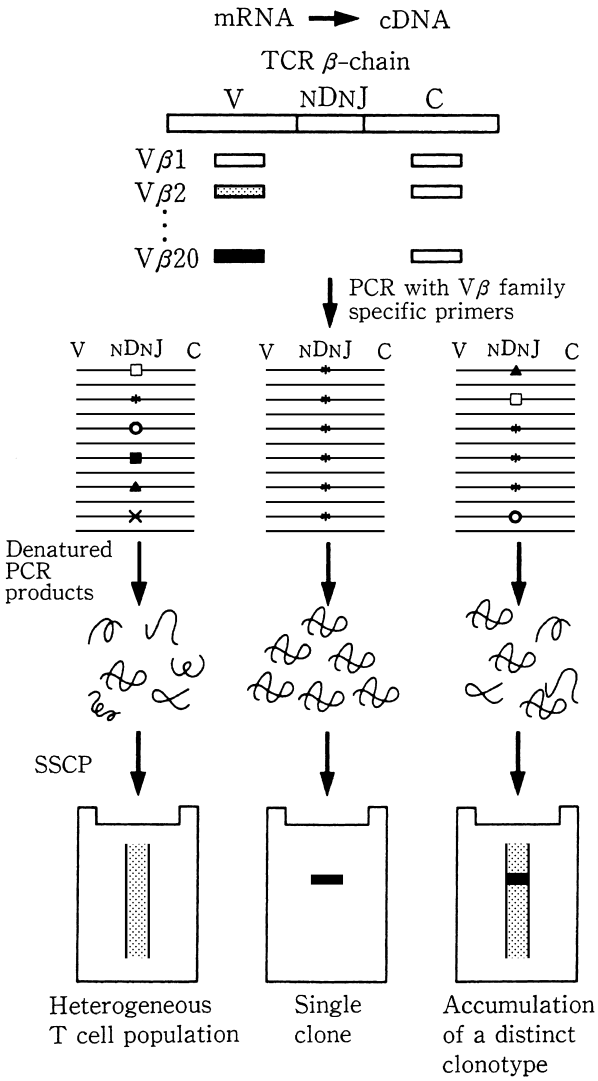

Fig. 1 Schematic diagram of the T cell clonality analysis. TCR, T cell receptor; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

reactions of T cells are carried out at the level of clones. Thus, it is important to evaluate whether T cells are clonally accumulated in a lymphocyte population to determine the existence of antigen-specific T cell responses.

In contrast, it is important to notice that TCR display a high degree of cross-reactivity in their recognition. Therefore, cross-reactivity between autoreactive T cells and microbial antigens may facilitate the development of autoimmune diseases.[4] For example, a recent study of Lyme disease suggests that this is an autoimmune process against the lymphocyte function-associated antigen (LFA)-1 protein expressed on activated T cells triggered by cross-reactive activation by *Borrelia burgdorferi* outer surface protein A.[5]

Establishment of a T cell clonality analysis system

We have established a novel method for analyzing T cell clonality using reverse transcription–polymerase chain reaction (RT-PCR) of TCR messages containing the most variable area, called the CDR3 region, and subsequent electrophoretic separation of the PCR product based on their single-strand conformation polymorphism (SSCP)[6] (Fig. 1). Using this method, an analysis of peripheral blood lymphocytes (PBL) obtained from a healthy individual revealed that each of the PCR products representing each of BV1 to BV20 exhibited a smear-like pattern. This pattern reflects the heterogeneity of the CDR3 region of the TCR β messages. We then stimulated the heterogeneous PBL with a mitogen, such as concanavalin A (Con A) or phytohemagglutinin (PHA). Such stimulation did not change the smear-like pattern. In contrast, when PBL were cultured in the presence of the tuberculin antigen purified protein derivative (PPD), several distinct bands appeared in the background smear pattern, indicating that the clones corresponding to these bands had been activated and had proliferated in the culture. A time-course analysis revealed that the number of these bands increased progressively during stimulation. $CD4^+$ clones were detected at first and CD8 clones then appeared to accumulate. We also followed the changes in the clonality of PBL *in vivo* before and after a flu-like infection. Accumulation of distinct T cell clones was again observed. Considering these findings, it is obvious that this method allows us to monitor the dynamic changes in accumulating T cell clones during antigen-specific immune reactions.

T cell clonal analyses of autoimmune diseases

Rheumatoid arthritis (RA) is believed to be mediated by autoimmune reactions, but it remains controversial as to whether T cells are really involved in the pathogenesis of RA. We analyzed T cell clonality in synovial samples obtained from RA patients during therapeutic synovectomy. Analysis showed that even though there were distinct clonal accumulations of T cell clones in the PBL of RA patients compared with healthy individuals, the synovial tissue samples contained even more distinct

accumulations of T cell clones. This indicates that there are antigen-specific T cell reactions in RA lesions. We then compared the T cell clonality among different joint lesions. With the SSCP system, identical clones generate bands that migrate to the same position in the gel. Thus, the identity of the clones could be compared when the PCR products from different samples were analyzed in adjacent lanes of the same gel. We found 70–90% of the accumulating T cell clones identified in one joint lesion also existed in other joints. This suggests that the same pathogenic immune reactions occur in multiple joints. In addition, the samples that were analyzed in our study represented rather advanced stages of RA. This suggests that pathologic lesions in advanced stages of RA are mediated by antigen-specific T cells.[7]

In addition, PBL from SLE were analyzed. Systemic lupus erythematosus patients in remission showed only a slight clonotypic expansion of T cell clones. However, PBL from patients with active SLE possessed a number of dominant clonal accumulations. Following administration of a high dose of steroids, these patients became inactive and the accumulated T cell clones in the PBL disappeared. Therefore, the degree of T cell clonal accumulation appears to correlate with the degree of disease activity.

Analysis of an organ-specific autoimmune disease also revaled similar results. For example, in patients with Graves' disease, accumulation of identical T cell clones in the right and left lobes of the thyroid gland was demonstrated, suggesting that there are immune responses driven by factors common to both lobes, such as thyroid-specific antigens.

During these analyses, we have found that T cell clones accumulated in lesions obtained from advanced phases of a disease were not necessarily large in number compared with those found in the relatively early phases. This finding does not fit with the idea of 'epitope spreading' recently proposed for T cell activation behavior in the autoimmune process.[8] According to this 'epitope spreading', the autoimmune epitopes may be limited in the initiation phase of the disease, but those recognized by T cells should spread during disease development and multiple T cell clones recognizing multiple autoepitopes should be activated in the relatively late phase of the disease. In order to know the real changes in activated T cell clones during the autoimmune process, we are now examining several models of spontaneous autoimmune mice. Our results so far do not support the 'epitope spreading' concept and, instead, suggest that T cell clones accumulated in the late phase of the disease are rather restricted and appear to play important roles in the pathogenesis. Thus, these T cells should be the target of therapy in the future.

Identification of target antigens recognized by accumulated T cell clones

To date, there are only a few methods available to identify the target antigens recognized by accumulated T cell clones. These include establishing T cell lines, stimulation with putative antigens and measurement of cell proliferation as a total lymphocyte population and the antigen–MHC tetramer technique. However, none of these methods can be applied in many situations. In this regard, we are now expanding our system of T cell clonal analysis to determine the nature of a specific target antigen. As described above, the same clones in different samples are easily identified by analyzing the PCR products in the same electrophoresis gel. Therefore, we can compare, for example, *in vivo* accumulating T cell clones in a pathologic lesion and T cell clones generated by *in vitro* stimulation of PBL with a putative antigen.

In conclusion, antigen-specific T cell clones appear to play important roles not only in organ-specific autoimmune diseases, but also in systemic autoimmune diseases. T cell clones accumulated in a relatively advanced disease phase may be qualitatively different from those found in the early phase. These T cells in both phases may be related to the pathogenesis in different ways and, thus, further investigations are necessary. Moreover, it is obvious that some of these clones can be used as the candidate targets of future immunotherapy.

References

1 Ji H, Korganow AS, Mangialaio S *et al*. Different modes of pathogenesis in T-cell-dependent autoimmunity: Clues from two TCR transgenic systems. *Immunol. Rev.* 1999; **169**: 139–46.
2 Ridgway WM, Fasso M, Fathman CG. A new look at MHC and autoimmune disease. *Science* 1999; **284**: 749–51.
3 Eisenberg R. Mechanisms of systemic autoimmunity in murine models of SLE. *Immunol. Res.* 1998; **17**: 41–7.
4 Albert LJ, Inman RD. Molecular mimicry and autoimmunity. *N. Engl. J. Med.* 1999; **341**: 2068–74.
5 Hemmer B, Gran B, Zhao Y *et al*. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nat. Med.* 1999; **5**: 1375–82.

6 Yamamoto K, Masuko-Hongo K, Tanaka A *et al*. Establishment and application of a novel T cell clonality analysis using single-strand conformation polymorphism of T cell receptor messenger signals. *Hum. Immunol.* 1996; **48**: 23–31.
7 Ikeda Y, Masuko K, Nakai Y *et al*. High frequencies of identical T cell clonotypes in synovial tissue of rheumatoid arthritis patients suggest the occurrence of common antigen-driven immune responses. *Arthritis Rheumatol.* 1996; **39**: 446–53.
8 McCluskey J, Farris AD, Keech CL *et al*. Determinant spreading: Lessons from animal models and human disease. *Immunol. Rev.* 1998; **164**: 209–29.