The cause of systemic lupus erythematosus: implication of ‘self-organized criticality theory of autoimmunity’ on the pathogenesis of systemic lupus erythematosus

To determine the cause of autoimmune disease, instead of chasing a causative autoantigen or seeking the cause of autoreactivity, we have studied the integrity of the immune system by stimulating the system maximally with an antigen (i.e., providing an external disturbance, just like testing the capability of an automobile in an extreme condition like an F1 race). Our research suggests that systemic autoimmunity (i.e., systemic lupus erythematosus) is the inevitable consequence of overstimulating the host’s immune system with an antigen to levels that surpass the system’s stability limit (i.e., self-organized criticality), a theory we call the ‘self-organized criticality theory’ of autoimmunity. We show that autoantibodies are not induced by cross-reaction to the antigen, but by de novo T-cell receptor revision giving rise to a novel T-cell type we term an autoantibody-inducing CD4 T cell (aiCD4 T cell). This aiCD4 T cell helps B cells to induce a variety of autoantibodies and facilitates the generation of effector CD8 T cells (i.e., cytotoxic T lymphocytes) via antigen cross-presentation, leading to immune tissue injuries. Thus, the cause of systemic lupus erythematosus depends on the robustness (i.e., the stability) of one’s immune system to present and/or cross-present the antigen to the T cells. It follows that the ability of microorganisms to cause autoimmunity depends on their propensity to be presented and/or cross-presented effectively, since we are normally exposed to a variety of environmental antigens but clinical features are masked upon subsequent infection owing to nonresponsiveness of previously activated effector cytotoxic T lymphocytes.

KEYWORD: antigen cross-presentation, antigen presentation, autoimmune disease, self-organized criticality, SLE, systemic lupus erythematosus

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The concept of ‘autoimmune disease’
The famous ‘clonal selection theory of acquired immunity’ proposed by Macfarlane Burnet and the subsequent molecular biological discovery of V(D)J recombination underlying the diversity and individuality of the immune response have revolutionized the concept of immunology in the last century. The concept of ‘autoimmune disease’ that was subsequently proposed has also been widely accepted by the scientific community as it appeared to explain the phenomena that have been termed ‘autoimmunity’ (Figure 1) [1]. However, recent studies show that autoimmune phenomena and autoantibodies are found not only in autoimmune diseases, but may even be found for some normal immune responses [2,3].

We have studied the pathogenesis of autoimmunity from a different angle, testing the integrity of the immune system. The approach we used was to repeatedly immunize mice normally not prone to autoimmune disease. Much to our surprise, we discovered that repeated immunization reproducibly led to the development of systemic autoimmunity (i.e., systemic lupus erythematosus [SLE]) [4]. We have accordingly proposed that systemic autoimmunity develops when immunostimulation exceeds some critical limit in the homeostatic capacity of the immune system, a theory we call the ‘self-organized criticality theory’ of autoimmunity. Our studies have revealed that autoantibodies are not induced by cross-reaction to an antigen, but by de novo T-cell receptor (TCR) revision (i.e., V(D)J recombination) at peripheral immune organs, such as the spleen, giving rise to a novel T-cell type we term an autoantibody-inducing CD4 T cell (aiCD4 T cell) (Figure 2). We have found that aiCD4 T cells help B cells to induce a variety of autoantibodies and facilitate the generation of fully mature CD8 T cells (i.e., effecter cytotoxic T lymphocytes [CTLs]) via ‘antigen cross-presentation’, thus leading to immune tissue injuries.

These findings are consistent with the current consensus that CD4 T cells normally die via activation-induced cell death after repeated exposure to a single antigen, while naive CD4 T cells with a ‘cross-reactive’ TCR and lower affinity can be activated through repeated exposure to the same antigen, and survive due to weak TCR signaling, ultimately acquiring autoreactivity [5]. However, the difference is that aiCD4 T cells are induced not by cross-reaction, but by de novo TCR revision, and the aiCD4 T cells thus generated induce systemic autoimmunity (i.e., SLE) in our study.
Figure 1. Difference between infection and Mackey’s autoimmune disease theory. When infectious antigens are removed in an infection, antigen-reactive CD4 T cells normally die via AICD. Without the continued help of CD4 T cells, B cells stop producing antibodies and CD8 T cells become anergic (i.e., they enter a state of antigen-induced nonresponsiveness), and do not exert cytotoxic effects, therefore the immune tissue injury ceases. What is called ‘autoimmunity’ will activate CD4 T and B cells to induce autoantibodies and tissue injuries in Mackey’s theory.

AICD: Activation-induced cell death; AINR: Activation-induced nonresponsiveness.

Self-organized criticality theory explaining the cause of SLE

Our self-organized criticality theory explains that systemic autoimmunity necessarily takes place when the host’s immune system is overstimulated by repeated exposure to an antigen at levels that surpass the immune system’s self-organized criticality [4]. The cause of SLE is thus not complicated from the perspective of systems biology. The specific causative antigens may be individually different, but each antigen must possess the following characteristics: it must overstimulate the person’s CD4 T cells in relation to his/her HLA [4], and it must overstimulate CD8 T cells to fully mature into CTLs via antigen cross-presentation with the help of αCD4 T cells [4]. Thus, the propensity to develop SLE depends basically on one’s genetically determined, or sometimes functional, ability to present and/or cross-present antigens to T cells. It then follows that the ability of a certain antigen, for example measles virus, to cause autoimmunity is due to its propensity to be presented and/or cross-presented effectively, resulting in the overstimulation of CD4 and/or CD8 T cells of certain hosts beyond their critical limit (i.e., self-organized criticality). Living organisms are constantly exposed to a broad range of environmental antigens, as exemplified by the recent re-emergence of measles virus infection among a subpopulation of Japanese young adults who
were not vaccinated against the virus. We must note that often the clinical manifestations of such repeated exposure do not appear immediately due to depletion of available antigen-specific effector CTLs. This is an attribute of CTLs that goes into memory (i.e., activation-induced nonresponsiveness) [6,7] when an invasive antigen is eliminated. Thus, the development of clinical symptoms following reinfecion may require that CTLs are first reactivated.

Why is evidence of cross-reactivity so frequently seen in studies of autoimmune diseases? One possibility would be that while immature lymphocytes in the thymus or bone marrow can proliferate autonomously, mature lymphocytes residing in peripheral organs do not proliferate spontaneously and thus require antigenic stimulation to proliferate. For this reason, the V(D) J recombination taking place at the periphery must be antigen driven, and, therefore, cross-reactivity can be observed: this cross-reactivity is in fact the reactivity against inducing antigen. Autoimmunity can be triggered by many environmental factors, among which infectious agents are pivotal [3,8]. We found that the TCR-β chain is never revised despite frequent TCR-α revision after repeated immunization with an antigen [Uto K, Tsumiyama K, Shiozawa S: The de novo TCR revision is responsible for generating autoantibody-inducing CD4+ T cells (aiCD4 T cells) that causes systemic autoimmunity, Manuscript in Preparation]. We speculate that an exogenous antigen can be a driving force for the selection of the TCR-β chain. The rearrangement of the TCR-β chain is inhibited due to histone modification, changes in locus conformation or subnuclear segregation of the corresponding TCR-β genes [9,11]. By contrast, the TCR-α chain can be rearranged to acquire autoreactivity rather freely during this time [4]. Furthermore, while females are more prone to develop SLE than men, a study has shown that repeated stimulation may cause different and stronger T-cell responses in women than in men [12].

**Lupus tissue injuries caused by fully mature CD8 T cell (effector CTLs)**

Balb/c strain mice are not prone to spontaneous autoimmune diseases. However, when subjected to repeated immunization with antigens such as ovalbumin (OVA), they develop autoimmune disease characterized by the presence of autoantibodies and immune tissue injuries, including immune complex-deposited membranous and/
or proliferative glomerulonephritis, a positive skin lupus band test, and lymphocyte infiltration into the salivary gland, bile duct, thyroid and skin (Figure 3A) [4]. The ability to induce autoantibodies and/or develop tissue injuries can be 100% reproducibly transferred to naive recipients via fully mature CD4 T cells and/or effector CD8 T cells, respectively (Figure 3B) [4].

The observation that CTLs can induce tissue injuries is not novel. However, the observation that both antigen cross-presentation [4] and aiCD4 T cells, but not antigen-specific CD4 T cells [13,14], are required for the full maturation of CTLs is a recent finding [4], and provides a new context for the role of antigen cross-presentation (Figure 4) [4]. In

**Figure 3. Induction of systemic lupus erythematosus.** (A) Upon repeated immunostimulation with an antigen such as OVA, 100% of mice developed autoimmune diseases characterized by the presence of a variety of autoantibodies and immune tissue injury, including immune complex-mediated glomerulonephritis and a positive skin lupus band test. Importantly, our immunization was administered intraperitoneally or intravenously and we did not use adjuvants, because only partial autoimmune-like pictures can also be induced by adjuvants [23]. Individual plots represent individual mice (mean ± standard deviation). (B) The ability to induce autoantibodies and immune tissue injuries (i.e., represented here by proteinuria) can be transferred into naive recipients via CD4 T cells and CD8 T cells, respectively. Note that B cells cannot induce autoantibodies or tissue injuries. Individual plots represent individual mice (mean ± standard deviation).

Ab: Antibody; H&E: Hematoxylin and eosin; OVA: Ovalbumin; PBS: Phosphate-buffered saline; RF: Rheumatoid factor.

Reproduced with permission from [4].
SLE, IFN-γ-producing effector CTLs and CD4 CD8 double-negative T cells are characteristically increased in sera and renal tissues [15–17]. These double negative T cells may represent overstimulated CD8 T cells [17] or natural killer T cells that are induced by IFN-α stimulation [Akiyama C, Uchimura C, Hashiramoto A et al.: IFN-α causes systemic lupus erythematosus. Manuscript in Preparation]. The IFN-α-producing aiCD4 T cells may thus provide the third signal postulated by Mescher and others required for the full maturation of CD8 T cells [6].

**Figure 4. Autoantibody-inducing CD4 T cells help maturation of CD8 T cells into cytotoxic T lymphocytes.** In mice immunized eight times with OVA, OVA-reactive CD4 T cells are erased by anti-CD4 T antibody treatment, fully matured CTLs do not develop and remain a precursor CTL. However, if aiCD4 T cells are generated in mice immunized 12 times with KLH, transferred CD4 T cells help the maturation of OVA-reactive CD8 T cells into effector CTLs. The results show that both OVA-specific IFN-γ-producing activated CTLs and immune tissue injury arose in these mice, indicating that aiCD4 T cells with de novo T-cell receptor revision are required to support the full maturation of CD8 T cells into CTLs, thereby generating immune tissue injury.

aiCD4: Autoantibody-inducing CD4; CTL: Cytotoxic T lymphocyte; KLH: Keyhole limpet hemocyanin; OVA: Ovalbumin; PBS: Phosphate-buffered saline; pCTL: Precursor CTL.

Reproduced with permission from [4].

SLE is caused by aiCD4+ T cells

Normally, once invasive antigens are eliminated during the course of infection, previously activated CD4 T cells die via activation-induced cell death, and CD8 T cells survive by becoming anergic, a state called antigen-induced non-responsiveness (Figure 1) [6,7]. However, in SLE, some CD4 T cells appear to survive beyond activation-induced cell death and continue to help CD8 T cells to mature into effector CTLs [5]. We propose that these could be the aiCD4 T cells. The ability of aiCD4 T cells to promote maturation can be tested by transferring CD4 T cells from mice immunized 12 times with keyhole limpet hemocyanin into the CD4 T-cell-depleted Balb/c mice immunized eight times with OVA in which OVA-reactive CD8 T cells are still immature (Figure 4) [4]. The result shows that both OVA-reactive effector CD8 T cells and immune tissue injury are generated in these mice, which suggests that aiCD4 T cells with de novo TCR revision are generated and helped precursor CTLs to mature into effector CTLs (Figure 4). In fact, we found that upon repeated immunization with antigen, the once anergized CD4 T cells recover from anergy and simultaneously produce a variety of autoantibodies including IgG and IgM rheumatoid factors, rheumatoid factor reactive against galactose-deficient IgG typically found in human autoimmunity, and anti-Sm and anti-dsDNA autoantibodies [4]. The TCR-α chain of CD4 T cells undergoes V(D)J gene rearrangement (i.e., TCR revision), thereby generating aiCD4 T cells capable of inducing autoantibodies from B cells [18]. The ability of aiCD4 T cells to induce autoantibodies were 100% transferable into naive recipients by the transfer of CD4 T cells (Figure 3B) [4]. Such somatic mutations often occur in lymphocytes.
and somatic mutation represents one of the major stochastic elements in the pathogenesis of autoimmunity [19]. The transfer of B cells, however, cannot induce autoantibodies or immune tissue injury (Figure 3B) [4]. This suggests that B cells play passive roles and the T-cell-centered regulatory mechanism is fundamental for the development of autoantibodies. This is compatible with the findings that many pathogenic anti-DNA antibodies are the products of a germinal center reaction: they exhibit heavy chain class-switching and have undergone somatic hypermutation [20].

**Generalization of the self-organized criticality theory**

We have demonstrated that aiCD4 T cells, induced by de novo TCR revision, can cause systemic autoimmunity (i.e., SLE) [4]. The induction of aiCD4 T cells is a critical first step, and the subsequent induction of effector CTLs by aiCD4 T cells is the next step in the development of SLE.

Systemic lupus erythematosus is unique among rheumatic diseases in the great variety of autoantibodies generated [21], which is in line with our finding that generation of aiCD4 T cells is sine qua non for the induction of a variety of autoantibodies [4]. However, it seems unlikely that aiCD4 T cells play a major role in other rheumatic diseases where only a few autoantibodies are produced. In other rheumatic diseases, CD4 T cells do not seem subject to the same degree of hyperstimulation required to mature into aiCD4 T cells. This theory, however, can be tested by marking aiCD4 T cells with a novel CD number [Miyazaki Y et al., Manuscript in Preparation]. Therefore, if the phenomena described here are correct, the concept of autoimmune disease or the mechanism underlying ‘autoimmune disease’ will require reconsideration, much as diseases such as gout, pseudogout or Becet’s disease were rationally reclassified under a novel unifying concept of autoinflammatory disease [22].

**Future perspective**

Most great discoveries are re-written by following students. One may take this as impolite or disrespectful, but to me, such revision is really proof that authentic research remains important. I have long respected Sir McFalane Burnet since my student days; however, as was the case for gout, pseudogout or Becet’s disease, which has been rationally reclassified under a novel unifying concept of autoinflammatory, but not autoimmune, disease [22], the concept of autoimmune disease or the mechanism underlying autoimmune disease now requires reconsideration rather than modification. If our self-organized criticality theory of autoimmunity is correct, the classification of autoimmune diseases will change. Our initial understanding is that according to this new paradigm, rheumatoid arthritis may not be an autoimmune disease, but rather an extreme example of an inflammatory cytokine disease that does not require the generation of aiCD4 T cells. We have preliminary evidence indicating that aiCD4 T cells exist in human SLE but not in human rheumatoid arthritis.
Implication of ‘self-organized criticality theory of autoimmunity’ on SLE

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I deeply thank my teacher the late A

Acknowledgements

Arthritis

Systemic autoimmunity, Manuscript in Preparation

Bibliography

PeRspective

Living organisms are self-organized systems (i.e., self-organized criticality).

The stability (i.e., robustness) of the host’s capabilities (i.e., genetic variation) to present and/or cross-present antigen to T cells. It then follows that the ability of any antigen, for example measles virus, to cause autoimmunity is due to their propensity to be presented and/or cross-presented effectively, thereby overstimulating CD4 and/or CD8 T cells of certain hosts beyond their critical limit (i.e., self-organized criticality).

The cause of SLE is not complicated or mysterious if one regards the causative antigen as just an antigen (i.e., external disturbance) that externally disturbs the stability of the host’s immune system, rather than pursuing a particular causative antigen common to every SLE patient.

The stability (i.e., robustness) of the host’s immune response as a ‘system’, rather than the chemical or biochemical nature of the causative antigens, is important in the pathogenesis of SLE.

Living organisms are constantly exposed to a broad range of environmental antigens, and repeated immunostimulation with an antigen is by no means exceptional in the real world. However, the clinical symptoms of such overstimulation often do not manifest owing to depletion of antigen-specific effector cytotoxic T lymphocytes.

Executive summary

- Repeated antigen immunization of mice not prone to autoimmune disease reproducibly leads to the development of systemic autoimmunity (i.e., systemic lupus erythematosus [SLE]).
- Systemic autoimmunity develops when immunostimulation exceeds some critical limit in the homeostatic capacity of the immune system, a theory we call the ‘self-organized criticality theory’ of autoimmunity.
- Autoantibodies are induced not by cross-reaction to the antigen, but by de novo T-cell receptor revision giving rise to a T-cell type we term an autoantibody-inducing CD4 T cell (aCD4 T cell).
- aCD4 T cells induce a variety of autoantibodies and facilitate the generation of fully mature CD8 T cells (i.e., effector cytotoxic T lymphocytes) via antigen cross-presentation, leading to immune tissue injuries.
- Causative antigens can be different, but they must fulfill the following characteristics:
  - They must overstimulate the person’s CD4 T cells in relation to his/her HLA.
  - They must overstimulate CD8 T cells to fully mature into cytotoxic T lymphocytes via antigen cross-presentation with the help of aCD4 T cells.
- The cause of SLE depends on the robustness of the host’s capabilities (i.e., genetic variation) to present and/or cross-present antigen to T cells. It then follows that the ability of any antigen, for example measles virus, to cause autoimmunity is due to their propensity to be presented and/or cross-presented effectively, thereby overstimulating CD4 and/or CD8 T cells of certain hosts beyond their critical limit (i.e., self-organized criticality).
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- The stability (i.e., robustness) of the host’s immune response as a ‘system’, rather than the chemical or biochemical nature of the causative antigens, is important in the pathogenesis of SLE.
- Living organisms are constantly exposed to a broad range of environmental antigens, and repeated immunostimulation with an antigen is by no means exceptional in the real world. However, the clinical symptoms of such overstimulation often do not manifest owing to depletion of antigen-specific effector cytotoxic T lymphocytes.

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arthritis [Uto K, Tsumiyama K, Shiozawa S: The de novo TCR revision is responsible for generating autoantibody-inducing CD4+ T cells (aCD4 T cells) that causes systemic autoimmunity. Manuscript in Preparation].

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