



Self-organized criticality theory and the expansion of PD-1-positive effector CD4 T cells: search for autoantibody-inducing CD4 T cells

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SELF-ORGANIZED CRITICALITY THEORY AND THE CAUSE OF SLE

The cause of systemic lupus erythematosus (SLE) remains unknown (Fu et al., 2011; Perry et al., 2011). A critical question in elucidating the pathogenesis of SLE or autoimmunity would be how autoreactive clones emerge and expand in the host. According to the prevailing view of autoimmune disease, autoreactive clones may come from either clones that had escaped negative selection in the thymus or clones that have been reactivated from tolerance. However, clones that would emerge by either process would be restricted in their antigen specificity and apparently could not account for the broad T cell receptor (TCR) repertoire and the more than 100 distinct autoantibody specificities found in SLE (Shiozawa, 2012).

We have subsequently developed an alternative novel theory, called the self-organized criticality theory, which proposes that autoreactive lymphocyte clones are newly generated *via de novo* TCR revision from non-autoreactive clones at peripheral lymphoid organs (Tsumiyama et al., 2009). We identified a CD4 T cell subset that has passed through TCR α but not TCR β revision at peripheral lymphoid organ spleen, and we named this an “autoantibody-inducing CD4 T cell” (*aiCD4* T cell).

The term “self-organized criticality” is derived from systems engineering. The theory proposes that systemic autoimmunity or SLE, necessarily takes place when the host’s immune system is overdriven by repeated exposure to antigen, reaching levels that surpass the immune system’s stability-limit, i.e., self-organized criticality

(Tsumiyama et al., 2009; Shiozawa, 2011, 2012). In our experiments, the novel T cells that result from such overstimulation, i.e., *aiCD4* T cells, not only could induce B cells to generate a large variety of autoantibodies, but also promote final differentiation of CD8 T cells into cytotoxic T lymphocytes (CTL) *via* antigen cross-presentation, leading to the tissue injuries identical to those seen in SLE (Tsumiyama et al., 2009). Thus, this *aiCD4* T cell is functionally indispensable for the pathogenesis of SLE. However, a precise characterization of these cells is still in progress, and we report here our recent findings on the distinct cell surface markers that characterize and identify *aiCD4* T cells.

THE *aiCD4* T CELL AND PD-1-POSITIVE EFFECTOR CD4 T CELL

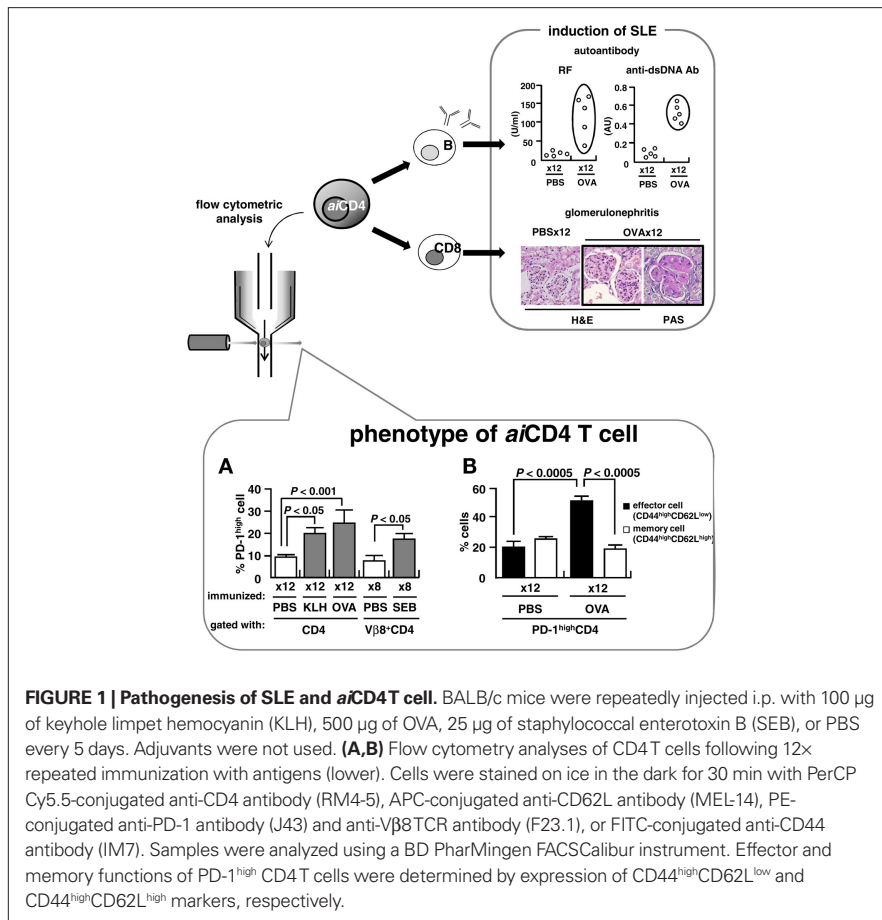
To further characterize the surface marker of *aiCD4* T cell, we generated *aiCD4* T cells in BALB/c mice, a strain that is normally not prone to autoimmune disease: 8-week-old BALB/c mice were repeatedly immunized (12 \times) with 100 μ g KLH (Sigma, St. Louis, MO, USA), 500 μ g OVA (grade V; Sigma), 25 μ g SEB (Toxin Technologies, Sarasota, FL, USA), or PBS by means of i.p. injection every 5 days. This protocol has been previously found to generate *aiCD4* T cells capable of inducing a variety of autoantibodies and pathological lesions identical to those seen in SLE (Tsumiyama et al., 2009). We found a significant expansion of CD4 T cells that expressed the PD-1^{high} marker (Figure 1A). These PD-1^{high} CD4 T cells further expressed CD44^{high}CD62L^{low} effector T cell markers, but not CD44^{high}CD62L^{high} memory T cell markers (Figure 1B). In

addition, these cells expressed CD27^{low}, CD45RB^{low}, and CD122^{high} markers (Miyazaki et al., 2013). We found that transfer of these PD-1-positive effector CD4 T cells into naïve recipients induced the generation of rheumatoid factor (RF) and anti-dsDNA antibodies. RF levels in the recipients were 39.36 \pm 10.92 U/ml (n = 3) vs. 11.38 \pm 5.21 U/ml (n = 7) in the controls (P < 0.0005), while anti-dsDNA antibody was 0.43 \pm 0.02 AU (n = 3) vs. 0.25 \pm 0.03 AU (n = 7) (P < 0.01), respectively.

Upon encounter with antigen, naïve CD4 T cells normally mature into CD27^{low}, CD127^{low}, CCR7^{low}, CD44^{high}CD62L^{low} effector cells (McKinstry et al., 2007; Wang et al., 2007). These effector cells subsequently differentiate into memory cells accompanied by increased expression of CD27, CD62L, CD127, and finally of PD-1 (Duraiswamy et al., 2011).

PD-1 belongs to the CD28 superfamily and is expressed on regulatory T cells (Treg) (Francisco et al., 2009), T follicular helper (Tfh) cells (Haynes et al., 2007), memory T cells, and exhausted CD8 T cells (Duraiswamy et al., 2011; Jin et al., 2011). PD-1 conveys a negative signal that causes reduced production of T cell cytokines, including IFN γ , TNF α , and IL-2 (Riella et al., 2012), and induces T cell tolerance (Jin et al., 2011; Riella et al., 2012).

We, however, saw no increase in FoxP3⁺CD25⁺ Treg upon repeated immunization with antigen in BALB/c mice. While CD25⁺CD4 T cells were increased in OVA-immunized mice as compared to PBS-treated mice; 80.1 vs. 68.3% (P < 0.01), the signal ratio of gene expression under



microarray analyses of the CD4 T cell of the mice immunized 12 \times with OVA relative to PBS-treated mice was 0.98 for FoxP3, 1.11 for Erg-2, and 1.29 for LAG3, and thus, the finding indicates that aiCD4 T cell is distinct from regulatory CD4 T subsets. Further, while Tfh markers, B cell leukemia/lymphoma 6 (Bcl6), inducible T cell costimulator (ICOS), and chemokine (C-X-C motif) receptor 5 (CXCR5), were also not significantly increased under microarray analyses, further studies should be required concerning the relationship between aiCD4 T cell and Tfh or exhaust T cell.

The cells that are expanded upon repeated immunization with antigen are the effector CD4 T cells uniquely expressing memory PD-1 marker. This effector CD4 T cell population would be unique in that despite expression of the PD-1 marker it shows increased production of IL-2 and IL-6 (Miyazaki et al., 2013). Production of IL-2 should normally be suppressed when PD-1 is expressed (Latchman et al., 2001; Riella et al., 2012), however, as shown by several studies, sufficiently strong signal-

ing through CD28 and/or IL-2 receptor can overcome PD-1 inhibitory signaling (Nurieva et al., 2006; Bertias et al., 2009). In fact, the PD-1-expressing CD4 T cell population which we found was significantly increased both in NZB/W F1 mice (Kasagi et al., 2010) and in the peripheral blood of patients with SLE (Liu et al., 2009). Since this CD4 T cells induce varieties of autoantibodies and pathological lesions identical to those seen in SLE upon transfer into naïve recipients (Tsumiyama et al., 2009), it appears that this PD-1-expressing effector CD4 T cell is an activated T cell type which has overcome PD-1 inhibition.

ROLE OF B CELL: IS T CELL-CENTERED DISEASE MECHANISM VALID?

While B cells produce autoantibodies, and intrinsic biochemical abnormalities in B cell, and T cell as well, can induce SLE, studies show that autoantibodies are produced from B cells that have undergone somatic hypermutation (SHM) with affinity maturation and class switching (Weinstein et al., 2012). Autoantibodies

arise predominantly from non-autoreactive B cells that diversify immunoglobulin genes *via* SHM: SHM normally diversifies antibody genes during physiological responses to foreign immunogen within the microenvironment of the germinal center and thus, autoantibody-producing B cells are generated *de novo* at periphery (Guo et al., 2010; Weinstein et al., 2012). Therefore, T cell-centered disease mechanism seems valid in the pathogenesis of systemic autoimmunity.

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