Viewpoint

Lymphopenia and autoimmune diseases

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Autoimmunity induced by lymphopenia

More than 30 years ago, the occurrence of spontaneous autoimmune thyroiditis was observed in rodents that were made severely T-cell lymphopenic by neonatal thymectomy or by thymectomy at week five after birth together with concomitant low dose irradiation [1,2]. Following these reports, numerous studies have shown that manipulations that generate functional T-cell lymphopenia result in the development of a variety of organ-specific autoimmune diseases in animal models (reviewed in [3]). Impressive examples of such manipulations include: IL-2 knockout mice, that develop prominent autoimmune colitis [4]; T-cell receptor-alpha chain deficient mice, that develop inflammatory bowel disease associated with an array of autoantibodies [5,6]; T-cell receptor-α chain transgenic mice [7]; neonatal application of cytotoxic intervention protocols, such as cyclosporine A [8]; total lymphoid irradiation [9] or thymectomy [10]; and lymphotoxic treatment of adult animals [11].

It was subsequently found that adoptive transfer of T cells into congenic immunocompromised hosts initiated the spontaneous development of aggressive inflammatory autoimmunity in recipients [12]. Further studies revealed that the development of autoimmunity in hosts was critically dependent on both transfer of alpha/beta CD4-positive T cells and T-cell deficiency in the recipients. Together, these data indicate that lymphopenia promotes the induction of autoimmune inflammation by self-reactive syngeneic peripheral blood CD4 T cells. Indeed it could be demonstrated that when lymphopenia was induced in mice by cytotoxic treatment with cyclophosphamide or streptozotocin, the peripheral T-cell population that emerged consisted mainly of IFN-γ secreting proinflammatory Th1-like cells [13]. However, it was unclear whether the appearance of these cells reflected *de novo* priming of autoreactive inflammatory T cells in the lymphopenic host or the preferential outgrowth of pre-existing T cells of autoimmune specificity, facilitated by a breakdown of suppressive mechanisms. Thus, the critical question for the understanding of autoimmunity, namely how the causative autoimmune response was initiated, remained unresolved.

Recent studies have shed light on the mechanisms that lead to the breakdown of peripheral tolerance in lymphopenic animals. Powrie and her group demonstrated that colitogenic inflammatory CD4 T cells exist in normal mice [14]. Importantly, their function is controlled in healthy animals by regulatory mechanisms involving IL-10 and a distinct subset of CD4 T cells characterized by the expression of CD25. Moreover, adoptive transfer of these CD25-positive CD4 T cells prevented T-cell-mediated immune pathology and even ameliorated established gastrointestinal inflammation in the CD4 $CD45RB^{high}$ T-cell transfer model of inflammatory bowel disease [15]. These findings emphasize that autoreactive T cells are part of the normal peripheral T-cell repertoire and that their control is an active process mediated by the CD25-expressing subset of CD4 T cells.

CD25-positive CD4 T cells with regulatory capacity have been described by Sakaguchi and colleagues as a population of thymus-derived CD4 T cells in the peripheral blood that prevents the occurrence of a variety of organ specific autoimmune diseases primarily affecting endocrine organs and the gastrointestinal tract [16]. CD25-positive T cells with regulatory capacity have therefore been denoted regulatory T cells (T_{regs}) or naturally occurring regulatory T cells.

Sakaguchi's group recently reported that CD25 T_{regs} can be characterized by the expression of the transcription factor Foxp3 and that retroviral expression of Foxp3 converts naive T cells towards a regulatory phenotype resembling that of naturally occurring T_{regs} [17]. Of interest, adoptively transferred Foxp3-expressing T cells

BMT = bone marrow transplantation; IFN = interferon; IL = interleukin; NOD = non-obese diabetic; SLE = systemic lupus erythematosus; Th = T helper; T_{regs} = regulatory T cells.

prevent autoimmune colitis and gastritis in the CD4 $CD45RB^{high}$ T-cell transfer model. These results clearly indicate that in healthy individuals a delicate balance exists between pathogenic autoreactive T cells and the regulatory T-cell population that keeps them in control. In lymphopenia this balance is perturbed and the outgrowth of autoantigen-specific, proinflammatory T cells is facilitated by the depletion of the regulatory T-cell subset.

A recent publication by Sarvetnick and her group now offers a distinct facet to the concept of controlling the emergence of autoreactive T cells in the periphery [18]. The authors observed that diabetes-prone non-obese diabetic (NOD) mice are lymphopenic and have reduced numbers of T cells compared to non-autoimmune strains, such as wild type BALB/c mice, NOD MHC-matched B10 mice and congeneic B6.Idd3.NOD mice that contain a 0.35 centimorgan protective interval from B6 mice and do not develop diabetes. They found that increasing T cell numbers in the mice by immunization with non-specific activators of the immune system, such as mycobacterial cell wall constituents (e.g. complete Freud's adjuvant), protected NOD mice from developing diabetes, indicating a correlation between increased T-cell numbers and disease protection. In accordance with this hypothesis, the injection of excess CD4 T cells from NOD mice into pre-diabetic NOD littermates prevented the development of diabetes in the recipients.

Injecting labeled T cells specific for pancreatic β cells into pre-diabetic NOD mice revealed that the transferred T cells vigorously proliferated in the lymph nodes of recipient NOD mice, but not in the lymph nodes of NOD mice that had elevated T cell numbers because of infusions of syngeneic excess T cells or immunization with complete Freud's adjuvant. The authors assessed cell surface receptors to distinguish between conventionally activated T cells and T cells that expand homeostatically. They demonstrated that a significant fraction of T cells in autoimmune NOD mice expand homeostatically and that the expansion correlates with autoimmune inflammation, e.g. lymphocytic infiltration of pancreatic islets. As homeostatic expansion is tightly regulated by the available space in lymphoid organs [19], the authors conclude from their data that a depleted memory T-cell compartment fuels the generation of autoreactive effector cells in lymphopenic diabetogenic NOD mice. The particular role of CD25-positive T_{regs} or other T cells with a regulatory capacity in those mice that had higher T cell numbers and did not develop diabetes was not specifically addressed in the study; however, the data suggest that organ-specific autoimmunity is initiated by lymphopenia and compensatory homeostatic expansion of autoreactive T cells.

Human autoimmune diseases and lymphopenia

Lymphopenia is not uncommon in several human autoimmune diseases. Reduced total lymphocyte counts are observed in rheumatoid arthritis, insulin-dependent diabetes mellitus, Crohn's disease, systemic lupus erythematosus (SLE) and primary vasculitides. Similarly, primary Sjogren's syndrome is associated with severe lymphopenia in 5% of patients, and the relative risk for CD4 T-cell lymphocytopenia in patients with Sjogren's syndrome has been estimated to be between 3.4 to 6000 [20,21].

Patients exposed to silica show a significant reduction of peripheral blood lymphocytes besides the well-established increased risk of autoimmune phenomena [22]. However, it is noteworthy that lymphopenia was observed in 48 out of 53 silicotic subjects while only 10% developed overt clinical autoimmune disorders [22]. Thus, other genetic or environmental factors are likely to have been involved in the development of autoimmune diseases in patients investigated in that study.

Lymphopenia constitutes one of the disease criteria in the American College of Rheumatology classification of SLE. It is, however, extremely difficult to determine whether lymphopenia is the cause or the consequence of systemic autoimmunity involving bone marrow in this context. Indeed the concomitant decrease in thrombocyte and erythrocyte blood counts in SLE patients might argue in favor of bone marrow deprivation as a result of lupus activity and against lymphopenia as the cause of the autoimmune inflammation.

Systemic inflammation *per se* affects peripheral blood cell counts, and increased numbers of circulating activated lymphocytes have been detected in almost every human autoimmune disease. Consequently, actual peripheral blood cell counts may reflect organ involvement in the underlying disease, systemic disease activity as well as immunosuppressive therapy.

The relationship between hematologic abnormalities and autoimmunity in humans was explored further by comparing patients with insulin-dependent diabetes mellitus and their first-degree relatives and healthy controls [23]. In contrast to *a priori* expectations, CD4 T-cell counts were normal in patients and significantly elevated in their non-diabetic first-degree relatives. Another argument against a causative role for lymphopenia in human autoimmune diseases derives from the observation that lymphopenia following infections with bone marrow-depriving viruses, malnutrition or drug-induced bone marrow toxicity is not commonly complicated by autoimmune manifestations.

The hypothesis that lymphopenia may not be sufficient for human autoimmune disease development is further supported by wide experience with ablative chemotherapy and autologous bone marrow transplantation (BMT) for malignant diseases. Although various autoimmune phenomena, including SLE, thyroiditis, thrombocytopenic

purpura, hemolytic anemia, Guillain-Barré syndrome, acute disseminated encephalomyelitis and myasthenia gravis [24,25], have been documented following autologous BMT, the incidence of such cases is rather low. In contrast, several reports have highlighted the beneficial effect of autologous BMT that was performed for the treatment of malignancies on coexisting autoimmune diseases [26,27]. It is evident from these observations, and BMT studies in animal models of autoimmune disease, that the best results with regard to clinical remission of autoimmune inflammation required the strongest lympho-myeloablative regimens [26]. Based on these studies, autologous BMT has now successfully been applied as therapy for several refractory autoimmune diseases [28]. The impressive results obtained with high dose lympho-myeloablative conditioning regimens are in accordance with the concept that autoimmune diseases are maintained by activated autoreactive T cells which have to be eliminated as thoroughly as possible to achieve complete and lasting remission.

Conclusion

As autoreactive T cells are part of the normal peripheral T-cell repertoire, it is conceivable that their expansion and activation *in situ* governs the development of autoimmune disease. Homeostatic expansion of autoreactive T cells is controlled in healthy individuals by space limitation, and their activation is regulated by specialized T-cell subsets. In lymphopenic animals, homeostatic expansion is enhanced as a mechanism of compensation, resulting in spontaneous autoimmune phenomena. Lymphopenia, however, although frequently associated with autoimmune diseases, appears not to be sufficient for the development of human autoimmunity, which may require additional environmental and genetic factors to progress to clinical disease.

Competing interests

None declared.

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