

Autophagic Behavior of T Lymphocytes in Systemic Lupus Erythematosus

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Commentary

The purpose herein is to provide an update on the role of autophagy dysregulation in systemic lupus erythematosus (SLE) focusing our attention on T lymphocytes.

Macroautophagy (simply called 'autophagy' here) is a genetically programmed process that requires the activity of autophagy-related gene (Atg) proteins [1,2]. Autophagy is a lysosome-mediated catabolic process characterized by sequestration of cytoplasmic material in double-membraned vacuoles, the autophagosomes, which ultimately fuse with lysosomes forming autophagolysosomes. These peculiar organelles are specifically devoted to the degradation and recycling of wasted or altered proteins and organelles [1]. On these bases, autophagy was defined as a promoter of cell survival under stressful conditions (e.g., nutrient depletion and oxidative stress) providing an alternative source of nutrients to the cell. Autophagy thus represents an alternative form of degradation of cell components that, in parallel with the proteasomal system, support changes in the metabolic requirements of the cell [1]. The extremity of this catabolic process, i.e., when all the resources are exhausted, has been described to paradoxically lead to cell death [3]. Autophagy has been shown to play important roles in various biological processes, particularly in the immune response [4,5]. It has diverse functions in innate immunity such as pathogen recognition, elimination of microorganisms, control of inflammation, and secretion of immune mediators [6]. Autophagy also contributes to adaptive immunity through diverse mechanisms, e.g., antigen processing for presentation by major histocompatibility complex (MHC) class II/ class I molecules and control of development and effector function of T and B lymphocytes [5,7]. Over recent years, perturbations in autophagy have been implicated in a number of diseases, including autoimmune diseases [8-10]. Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease of unknown etiology, more frequently observed in women than in men (the ratio of women to men is 7:1-10:1), and characterized by polyclonal autoantibody production, immune complex formation and deposition in different parts of the body, leading to inflammation and multi-organ injuries [11]. Aberrant T-lymphocyte homeostasis plays a key role in disease pathogenesis, and much work has been done so far to define the underlying factors/mechanisms of disrupted T-cell homeostasis and to identify possible ways to manipulate them pharmacologically [12]. Given the potential implications of autophagy in autoimmunity, it is rather surprising to observe that only few reports investigated the functional correlation between autophagy and SLE [13-16]. However, lymphocytes, although of great interest in translational medicine, represent a very complex cell type for the study of autophagy. In fact,

the study of these cells displays a series of pitfalls due, among others, to the existence of different cell subsets with specific features and functions. Moreover, the activation processes, which modify lymphocyte function, and the peculiar, very high nuclear/cytoplasmic ratio (i.e., a very small cytoplasmic milieu which does not facilitate cytopathological analyses), clearly complicate the matter. Lymphocytes need a strictly regulated metabolic extracellular environment and their activation depend on a precise cell remodeling that involves key metabolism-associated organelles, such as mitochondria, so that autophagic evaluations are quite arduous. The data obtained so far by us and others, both *ex vivo* in freshly isolated lymphocytes (basal autophagy) and *in vitro* under autophagy stimulation (autophagy susceptibility), indicated an apparent disparity of autophagic behavior in lupus T cells versus normal T cells [13-16].

Basal Autophagy

A first study by Gros et al. [16] revealed that T cells from two distinct lupus-prone mouse models, i.e., MRL (lpr/lpr) and (NZB/NZW) F1, exhibited high loads of autophagic compartments compared with control mice. Autophagic vacuoles were also found to be significantly more frequent in T cells from a small cohort of lupus patients compared with healthy controls and patients with other autoimmune diseases. Interestingly, this elevated number of autophagic structures was not distributed homogeneously and was highly expressed in some T cells only. A further study carried out by our group [13] showed higher autophagy levels in naive CD4⁺, but not CD8⁺, T cells from patients with SLE as compared with those from healthy donors. These results could explain the heterogeneous distribution of autophagic structures in lupus T cells, observed by Gros et al. [16]. Peripheral post-thymic expansion of naive CD4⁺ T cells has been hypothesized to be driven by self-peptides for the maintenance of T-cell immunity in adults [17,18] and an unregulated expansion of these autoreactive T cells has been suggested to contribute to the pathogenesis of autoimmune diseases [17]. Hence, in SLE, proliferating, autoreactive naive CD4⁺ T cells could undergo enhanced autophagy in order to supply their metabolic needs.

Autophagic Susceptibility

A significant disparity in autophagic propensity between lupus and control T lymphocytes has been revealed by *in vitro* studies. T lymphocytes from lupus-prone mouse models, i.e., MRL (lpr/lpr) and (NZB/NZW) F1, under phorbol myristate acetate/ionomycin stimulation, showed higher levels of autophagy as compared to those from control mice [16]. This result is at variance with what observed in patients with SLE in which T lymphocytes, under different autophagic stimulations, showed a resistance to autophagy induction as compared to healthy controls [13-15]. In particular, *in vitro* unresponsiveness to

autophagy induction was observed in SLE T lymphocytes cultured under growth factor deficiency [13], a condition that is known to induce metabolic impairment and trigger autophagy [19]. A mechanism contributing to autophagy resistance of SLE T lymphocytes has been revealed by the overexpression, at both mRNA and protein levels, of α -synuclein [15]. In fact, alpha-synuclein has been demonstrated to inhibit autophagy by reducing autophagosome formation at a very early stage [20]. On one hand, autophagy defect in SLE T cells appeared to be associated to an accumulation of α -synuclein aggregates, which require functional autophagy to be degraded; on the other hand, increased α -synuclein protein burden may impair autophagy, generating thus a bidirectional positive feedback loop. Even more interesting from a clinical point of view is the significant inverse correlation we found between α -synuclein and autophagy levels in T cells. This suggests that this molecule, representing an autophagy-related marker of peripheral blood T lymphocytes, could be taken into account as predictive biomarker for autophagy-modulating drug response [15]. In fact, drugs that are potentially modulate autophagy, such as the mTOR inhibitors (e.g., rapamycin and derivatives), are increasingly being used for therapeutic purposes in immune-mediated diseases, including clinical trials on patients with SLE [21-23]. There is therefore a growing need for identifying predictive biomarkers for the efficacy of these drugs. Another important mechanism in autophagy resistance of T cells in SLE could be the chronic exposure to autophagic stimuli leading to selection of autophagy refractory T lymphocytes. Interestingly, serum autoantibodies purified from SLE patients were able to induce autophagy in T cells from healthy controls [13]. These autoantibodies were found to react with the small GTPase family inhibitor D4GDI expressed at the lymphocyte surface [14]. Anti-D4GDI autoantibodies were present in about 50% of sera from SLE patients and, binding to their antigenic target, were suggested to trigger a series of intracellular changes, e.g. cytoskeleton remodeling, and induce autophagy. This autophagy ignition was apparent in T cells from healthy donors as well as from SLE patients lacking of serum anti-D4GDI autoantibodies whereas it was undetectable in T lymphocytes from patients displaying anti-D4GDI autoantibodies in their sera [14]. Hence, it was hypothesized that the chronic exposure to these autoantibodies could lead to the selection of autophagy-resistant T cell clones in certain patients [14] whose clinical features are nowadays under characterization in our laboratory. This autophagic defect could result in an overload of damaged mitochondria that release apoptogenic factors, generate reactive oxygen species, and increase apoptosis, commonly observed events in lymphocytes from SLE patients [12], in few words; it could exert a critical pathogenetic role. In particular, this autophagy/apoptosis imbalance could contribute to hematologic manifestations, such as lymphopenia and leukopenia, which were more frequently observed in patients exhibiting serum anti-D4GDI autoantibodies [14].

Accordingly, available literature data, summarized in figure 1, strongly support the role of autophagy as pathogenic determinant in SLE. As depicted above, constitutively higher levels of autophagy were detected in naive CD4⁺ T cells from SLE patients as compared to healthy donors. Conversely, autophagy resistance was found in T cells from patients with SLE. In this regard, differential *in vitro* responses of human and mouse T cells to autophagic stimuli should be underscored as a paradigmatic example of the difficulties occurring in the translation of preclinical studies into clinical testing. A further difficulty in translating these new findings to the bedside, is the fact that autophagy is a double-edge sword allowing on the one hand the

maintenance of cellular homeostasis, but on the other hand the survival of autoreactive cells. Thus, further studies are mandatory to better understand the actual role of autophagy in the onset and in the progression of the disease. Finally, gender-based evidence suggesting a role for sex hormones in modulating autophagy and a different propensity of cells from males and females to autophagy induction [24-29] opens new paths for a better comprehension of SLE pathogenesis and for the optimization of clinical management of this disease.

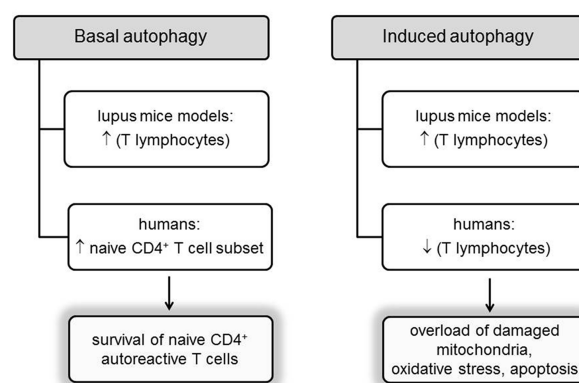


Figure 1

Figure 1: Defective autophagy in T lymphocytes from both lupus-prone mouse models and SLE patients are described both in freshly isolated lymphocytes (basal autophagy) and under autophagy stimulation (autophagy susceptibility).

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