Pathophysiological mechanisms of autoimmunity

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Short title: Introduction to Autoimmunity

PATHOPHYSIOLOGICAL MECHANISMS OF AUTOIMMUNITY

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Abbreviations:

AChR:	*acetylcholine receptor*
AID:	*autoimmune disease*
BAFF:	*B-cell activating factor*
eQTL:	*expression quantitative trait loci*
EOMG:	*early-onset myasthenia gravis*
GC:	*germinal center*
MG:	*myasthenia gravis*
Poly(I:C):	*polyinosinic-polycytidylic acid*
PTPN22:	*Protein tyrosine phosphatase, non-receptor type 22*
RA:	*Rheumatoid Arthritis*
SLE:	*Systemic lupus erythematosus*
TLR:	*Toll-like receptor*
TNF:	*Tumor Necrosis Factor*

Abstract

Autoimmune diseases (AIDs) are chronic disorders characterized by inflammatory reactions against self-antigens that can either be systemic or organ-specific. AIDs can differ in their epidemiologic features and clinical presentations, yet all share a remarkable complexity. AIDs result from an interplay of genetic and epigenetic factors with environmental components that are associated with imbalances in the immune system. Many of the pathogenic mechanisms of AIDs are also implicated in Myasthenia Gravis (MG), an autoimmune disease where inflammation of the thymus leads to a neuromuscular disorder.

The goal of this review is to highlight the similarities and differences between MG and other AIDs by reviewing the common transcriptome signatures and the development of germinal centers, and by discussing some unsolved questions about autoimmune mechanisms. This review will propose hypotheses to explain the origin of regulatory T (Treg) cell defects and the causes of chronicity and specificity of AIDs.

1. Introduction

Autoimmune diseases (AIDs) encompass more than 80 distinct chronic disorders characterized by inflammatory reactions that can either be systemic or organ-specific. Despite decades of research on AIDs and improvement of therapeutic approaches, their exact etiologies remain a conundrum. However, most recent findings suggest that AIDs result from the complex interplay of various factors (genetic, epigenetic, external factors as well as internal components under the influence of environmental factors). While these factors are not sufficient to induce AIDs when taken individually, their temporal combinations may pave the winding road to autoimmunity.

There are many similarities between the different AIDs, and polyautoimmunity is common in patients with AIDs and their families [1]. In all cases, the disease development is the consequence of the effects of environmental factors in predisposed individuals. Predisposing factors could be genetic or non-genetic. Various predisposing genes are shared between several AIDs, suggesting a partial redundancy between these different diseases. Most of the genes identified by genome-wide association studies on AIDs are related to immunity. However, functional immune parameters that are commonly dysregulated in AIDs do not necessarily stem from these genetic variants, as some of them may be directly induced by non-genetic (environmental) triggering events. This was notably illustrated in reports on monozygotic (MZ) discordant twins [2], showing that predisposing genes are not sufficient to develop an AID, and that environmental factors are needed. Environmental cues include predisposing factors (low vitamin D, smoking, pollution, sexual hormones) and triggering factors (specific drugs) as well [1]. Several factors frequently associated with AIDs are illustrated in a word cloud representation (**Figure 1**).

Many of the pathogenic mechanisms of AIDs are also implicated in Myasthenia Gravis (MG). Indeed, MG is multifactorial, and its onset is a consequence of interactions between various genetic and environmental risk factors. The goal of this review is to highlight the similarities and differences between MG and other AIDs such as the common transcriptome signatures, the development of GCs and the overexpression of BAFF. We will also discuss some unsolved questions about autoimmune mechanisms such as the origin of regulatory T (Treg) cell defects. Indeed, although defects of the

immune system are known for long whether they pre-exist in patients before the onset of the disease or are induced by triggering events deserves to be discussed. This review will also propose hypotheses to explain why AIDs are chronic and how the specificity of an AID is determined.

2. Typical molecular signatures in MG and other AIDs

At the molecular scale, transcriptomics has largely contributed to identifying pathogenic signatures in human autoimmunity. In early-onset myasthenia gravis (EOMG), the transcriptome of thymuses, which is the inflammatory organ in this disease, was compared with sex- and aged-matched non-MG thymuses [3] [4]. Briefly, the gene hit lists were established as reported [3] and submitted to David Bioinformatic resources [5]. Similarities were found in the category of "Genetic Association Database" that contains genetic association data from complex disorders. This analysis, shown in **Table 1**, involves several AIDs such as systemic lupus erythematosus (SLE), Sjogren's syndrome, thyroiditis or multiple sclerosis [4]. It is noteworthy that these similarities exist despite differences in the immunological mechanisms involved in these diseases [6].

A similar analysis of the transcriptome of purified thymic Treg and conventional T (Tconv) cells from MG patients in comparison with control cells reveals a 10-fold upregulation of genes known to be involved in other AIDs, such as Type 1 diabetes and autoimmune thyroiditis, and in pathological states associated with increased immune activation and inflammation, such as allograft rejection and graft-versus-host disease [7]. These data suggest that T cells contribute to the similarities between MG and the other AIDs.

The genes involved in the ten autoimmune diseases listed in Table 1 were analyzed to detect the genes shared between these disorders. We identified 16 genes dysregulated in the thymus of EOMG patients as well as in at least three other different AIDs (**Table 2**). These include many genes related to the immune response: HLA-DR and DQ, CD14 (monocytes), CD19 (B cells), CD80 (a costimulatory molecule), IL-1β (a cytokine) and CCL-5/RANTES (a chemokine), but also a gene involved in oxidative stress (NOS) and the protein tyrosine phosphatase, non-receptor type 22 (PTPN22). These

genes are expressed in other cells than T cells, suggesting that not only T cells but also other immune cell types are involved in MG. Most of these genes have a genetic association with MG [8]. The discovery that the level of expression of these genes is dysregulated in MG thymus suggests the presence of genetic regulators of transcription. Indeed, the combined analysis of transcriptomes and whole-genomes showed that the expression level of some genes could be regulated by DNA sequence variants localized in distinct genomic regions. These variants, named eQTL (Expression quantitative trait loci), provide a functional relevance for the association between genetic variants and AIDs. The eQTL analysis was recently applied to rheumatoid arthritis (RA) and successfully predicted the importance of the Tumor Necrosis Factor (TNF) pathway in CD4+ T cells [9]. Although eQTLs have never been studied directly for MG, a screen of cis-eQTLs for 353 AIDs risk variants in 42 human thymic tissues identified eight eQTLs associated with seven genetic regions including two regions that were thymus-specific [10]. Overall, rather than performing even larger GWAS, understanding complex traits such as human diseases may require the meticulous analysis or cell-specific gene networks and take into account not only core genes but also seemingly irrelevant genes that may overall have an impact on the disease [11].

Among the genes highly associated with AIDs, the most striking ones are HLA-DR1 and PTPN22. We will discuss here the potential role of PTPN22 as this gene is genetically linked to more than twenty AIDs [12], including MG [13]. PTPN22 is a potent inhibitor of the T-cell receptor signaling pathway [14]. In PTPN22 knock-out (KO) mice, effector-memory T cells cause lymphadenopathy, splenomegaly, and more germinal centers. High serum concentrations of antibodies are also found in these mice [15]. In humans, a single base change in the coding region of this gene (C1858T) is observed at a high frequency in patients with AIDs [16]. Consequently, the autoimmune disease incidence is 2.5-fold higher in carriers of the C1858T mutation compared to individuals without the mutation. This mutation is associated with a decrease in TCR and BCR signaling [17]. The expression of PTPN22 was found to be reduced in the MG thymus compared with controls, but the sequence of PTPN22 gene was not investigated in these samples. Akin to the data observed in PTPN22 KO mice

[18], the decreased expression of PTPN22 in the thymus of MG patients could be related to the increased B cell number and GCs.

3. Ectopic germinal centers and autoimmunity

In physiological conditions, B-cell activation and differentiation into antibody (Ab)-producing cells takes place in secondary lymphoid organs where activated B cells initiate a germinal center (GC) reaction and undergo the processes of somatic hypermutation and class switch recombination of the immunoglobulin genes. Follicular B cells require T cell help and are the primary source of long-lived plasma cells and memory B cells [19].

GCs generally develop ectopically in the target organ in antibody-mediated AIDs [20]. MG is an exception as the development of GCs is in the thymus, and not in the muscle that is the target organ (**Table 3**). GCs are found in many AIDs such as Sjogren's syndrome, RA, Hashimoto's thyroiditis, SLE, multiple sclerosis, primary biliary cirrhosis, as well as dermatomyositis and polymyositis (Reviewed in [6]). Surprisingly, the development of GCs in the thymus of MG patients is not a general MG feature as it occurs mainly in young female patients with anti-AChR antibodies [21], but not in patients with anti-Muscle Specific Kinase (MuSK) antibodies [22]. The reason for this difference is still poorly understood.

The generation of GCs is very similar in all AIDs as it is associated with chemokine overproduction (CXCL13, CCL21, CCL19, CXCL12)[23], and active angiogenesis involving high endothelial venules (HEVs) and lymphatic vessels [24] [25]. In the MG thymus, the HEVs likely contribute to the recruitment of B cells and antigen-presenting cells as these cells can be detected close to the HEVs [26]. The production of auto-antibodies resulting from GC development usually precedes the disease [27]. In MG thymuses, B cells appear to derive from an antigen-driven reaction [28]. These thymic B cells can produce antibodies to AChR, as assessed by titration in culture supernatants of thymic cells [29] or in the sera of immunodeficient mice grafted with MG thymic tissue [30, 31]. Importantly, anti-

AChR antibodies produced by MG thymic B cells are pathogenic since grafts of MG thymic fragments could result in myasthenic symptoms in immunodeficient mice [32].

The increased number of B cells in the MG thymus is associated with increased expression of BAFF, a factor supporting survival and differentiation of B cells [33]. Increased serum levels of BAFF are found in MG as well as in several AIDs [34]. Overexpression of BAFF in mice results in increased B cell numbers in spleen and lymph nodes, and a SLE-like autoimmune phenotype [35]. Inhibition of BAFF reduces the severity of the ongoing disease, supporting its pathogenic role [36]. In humans, an anti-BAFF monoclonal antibody (belimumab) has proven to be efficient in SLE and RA and is approved for treatment of SLE [21]. In MG, a phase II clinical trial using this monoclonal antibody is ongoing (ClinicalTrials. gov: NCT01480596).

Altogether, the development of GCs and the overexpression of BAFF are hallmarks of AIDs. The efficiency of anti-BAFF therapy in animal models and patients suggests a pathogenic role of BAFF in AIDs. Also, the presence of GCs in the pathogenic organs highlights its pivotal role in the recruitment of inflammatory cells and initiation and the propagation of the disease.

4. Origin of the defect of Treg cells

Regulatory T (Treg) cells are of paramount importance to prevent excessive inflammation in various organs. The lack of functional Tregs, in genetic diseases, including the scurfy mice and humans with the IPEX syndrome, leads to a broad range of inflammatory disorders affecting various tissues [37]. As such, Treg cell defects have been considered as a primary cause of AIDs. However, one could ask whether the Treg cell dysfunction exists before the onset of the disease, or is provoked by the inflammatory event induced by the triggering components. The defect of Treg cells generally coexists with the inflammatory processes suggesting several hypotheses: 1) the inflammation might develop because of a poor regulation of the immune system, 2) the Treg cells could become inefficient because of the inflammatory environment, 3) a common factor concomitantly leads to both effects.

According to the literature, several experimental findings support the hypothesis that Treg cell defects in AIDs could be a consequence, and not necessarily a primary cause, of the disease. We will summarize here these arguments:

1) A defect in Treg function can appear after immunization with an autoantigen in experimental autoimmunity. For example, in the EAMG rat model, the induction of the disease by immunization with torpedo-AChR results in a decreased frequency and suppressive activity of Treg cells in the spleen and peripheral blood compared with control rats [38].

2) The fact that Treg cell defect is often concomitant with a resistance of Tconv cells to suppression by normal Treg cells [39] suggests that the immune imbalance does not affect only the Treg cells, but more likely the whole immune system. The resistance of Tconv cells is likely due to the inflammatory microenvironment [40], and could be mediated by TNF-α. In MG cells, blocking TNF-α decreases the resistance of thymic Tconv cells to suppression [7], supporting the hypothesis that inflammatory molecules are, at least partially, responsible for the resistance of Tconv cells to suppression. In RA, T cells become resistant to regulation at the site of inflammation, and this contributes to the altered immune balance in the inflamed joints of RA patients [41].

3) Defects of Treg cells do not appear to be intrinsic as they can be reversed in vitro or in vivo. Anti-inflammatory drugs such as dexamethasone were shown to restore the function of Treg cells [42]. Other immunosuppressive drugs, such as rapamycin can have a marked effect on Treg cell number and function (reviewed in [43]). In many cases, as soon as AIDs patients are treated with anti-inflammatory drugs, the Treg cell function is improved. The commonly used corticosteroid drug improves Treg cell function in SLE [44], MG [45] and RA [46] patients. Intravenous immunoglobulins that are commonly administrated in patients with AIDs appear to act, at least partially, by restoring Treg cell function and number [47].

In MG animal models, Treg cell number and function are restored by therapeutic drugs. In mice, one example is the therapy by a peptide inhibitor of the plasminogen activator system in tissue plasminogen activator (TPA) -/- mice that have a severe MG and defects in Treg cell numbers [48]. Following the injection of the peptide inhibitor, the disease severity is reduced, and Treg cell numbers

are increased. A second example is a therapy via intravenous immunoglobulins or a subfraction including recombinant polyvalent IgG2a Fc (M045) in the classical model of EAMG induced by immunization with torpedo AChR. The M045 treatment improves the course of the disease and causes an expansion of the FoxP3+ Treg cell population [49].

Together, these data suggest that Treg cell defects in acquired AIDs are not necessarily pre-existing to the triggering event. Since the experimental triggering events (immunization for example) in vivo are different from the ones in natura, one major challenge will be to understand how predisposing factors (genetic, vitamin D level, hormones, microbiota, etc.) could contribute to the Treg cell defects in AIDs.

5. How to explain the specificity of autoimmune diseases?

Although frequent poly-autoimmunity is observed in families affected by AIDs, most often the patients have only one specific disease. Once the immune system is overactivated, one could ask why a patient will develop a particular disease and not another one.

Several non-mutually exclusive hypotheses could be proposed:

1) The role of HLA genes has been suspected for long. Each HLA molecule has a certain affinity for antigen-derived peptides. For instance, a peptide from an autoantigen will fit into the peptide-binding pocket of a particular HLA-DR allele, and the HLA-peptide complex will be recognized by the T-cell receptor, while other HLA-DR molecules would not bind the same autoantigenic peptide with a high affinity. However, this hypothesis does not explain why a certain HLA haplotype, e.g., HLA-DR3, is the susceptibility locus for several AIDs, including SLE, Type 1 diabetes, autoimmune thyroiditis, Sjogren's Syndrome and MG. One hypothesis could be that HLA-DR3 is more permissive than other HLA molecules to the presentation of in situ-generated hybrid molecules containing self-peptides, a phenomenon that was recently described for diabetes [50].

2) The nature of the triggering event could contribute to the specificity of the disease. Toll-like receptors (TLR) are involved in the recognition by the innate immune system of a broad range of

microbial ligands that can induce a disease in several experimental models of autoimmunity, including RA, diabetes, and atherosclerosis (Reviewed in [51]). It is worth-mentioning that some TLRs are more specifically associated with some AIDs. For example, TLR2 or TLR4 polymorphisms are frequently associated with RA, while TLR5 and TLR8 polymorphisms are associated with SLE and Sjogren's syndrome (Reviewed in [51]). Alternatively, regardless of TLR polymorphisms, qualitative environmental variations of TLR ligands may contribute to AIDs. For example, type 1 diabetes is associated with a specific lipopolysaccharide, the microbial TLR4 ligand [52]. In MG, we have shown that the TLR3 agonist poly(I:C), but no other TLR agonists, can upregulate the expression of α-AChR in thymic cells and induce myasthenic symptoms when injected into mice [53]. Interestingly, poly(I:C) does not affect the expression of other autoantigens, suggesting that poly(I:C) has a specific effect on AChR.

3) The nature of the organ affected by the triggering molecule is likely essential for the specificity of the autoimmune reaction. Infections can occur in any organ, but taking into account the cellular and molecular specificities of each organ, the functional consequences of a given perturbation could be very different. This assumption is particularly relevant in the perspective of the eQTLs that are genetic variants associated with the transcriptional expression of one or several genes. Indeed, more than 10% of eQTLs can be context specific, being selectively active in specific tissues or cell types [54]. A recent study found that about half of eQTLs are tissue-specific [55], highlighting the importance of considering tissues or cells of interest to understand how eQTLs may influence AIDs. Thus, a triggering event occurring in an organ that permits the excessive transcriptional expression of genes involved in the immune response could explain the targeting of this specific organ.

6. Why are AIDs chronic?

While AIDs are generally incurable chronic diseases, transient autoimmunity can also be swiftly induced by environmental triggers (notably drugs) and disappear upon their withdrawal. This fact indicates that chronicity is not a necessary consequence of autoimmunity. Many drugs can induce the

production of antibodies and autoimmune symptoms although the presence of autoantibodies is much more frequent than the appearance of symptoms (Reviewed in [56]).

In the case of SLE, a very common AID, a drug-induced disease was first described in 1945 after the treatment with sulfadiazine. Since then, a large number of drugs (more than 100) have been implicated in inducing lupus. Drug–induced SLE accounts for about 10% of all SLE cases and its presentation is similar to idiopathic SLE, but the symptoms usually resolve within weeks after drug withdrawal [57]. Thus, autoimmune diseases are not inevitably chronic. In drug-induced diseases, it is possible that the pathogenic effects are minimal as the disease is at an early stage. If this hypothesis is exact, an early diagnostic will provide a better chance of reversing the disease.

While the findings exposed above indicate that chronicity is not an inevitable consequence of autoimmunity, it is likely that autoimmunity results from a chronic imbalance involving both environmental and intrinsic factors. Several non-mutually hypotheses could explain the chronicity of the autoimmune response:

1) An ongoing antigenic availability linked to the maintenance of the GCs could account for the chronicity of the production of the autoantibodies. In physiological conditions, GC reactions disappear few weeks after their induction, while in AIDs these structures are very stable. For example, in AChR-MG patients, GCs could be found in the thymus of MG patients even ten years or more after disease onset, suggesting that B cells migrate continuously to the thymus [4]. Since the autoantigen (AChR) is expressed in several thymic cell types [58, 59] and could be upregulated by interferons [33], a high expression of the autoantigen will promote its availability. The stability of the GCs could be due to a defective mechanism of apoptosis within the GC. Indeed, the resolution phase of GCs is associated with high level of apoptosis [60]. While the anti-apoptotic factor Bcl-2 is not expressed in GCs of control tonsil, it is expressed in thymic GCs of MG patients where it may prevent cell apoptosis [61].

2) The resolution of inflammation that is a complex coordinated process to return to tissue homeostasis could be defective. Resolution of inflammation appears to be a bridge between innate and adaptive immunity. An incomplete resolution of the initial acute response that does not engage a proper adaptive immune response could lead to chronic inflammation [62]. Inflammation resolution

process is controlled by a variety of endogenous mediators that include specialized proresolving mediators such as lipoxins and resolvins, protein/peptide mediators, gases, as well as nucleotides [63]. As a proof of concept, in the murine model of autoimmune arthritis, the resolution of inflammation that is disrupted by cyclooxygenase-2 inhibition is restored by prostaglandin E2-mediated lipoxin A4 production [64]. In another model of murine inflammatory arthritis, the anti-arthritic properties of resolvin D1 could be demonstrated [65]. In MG, in a recent analysis of the transcriptome and methylome of peripheral monocytes in monozygotic (MZ) twins discordant and concordant for MG, we showed a decreased expression of several transcripts associated with immune homeostasis and inflammation resolution. Thus, the impaired monocyte function in MG and the decreased expression of genes associated with inflammation resolution could contribute to the chronicity of the disease. These examples show that the resolution of inflammation could be less efficient in AIDs patients than in other individuals, raising the susceptibility of developing a chronic disease.

3) Immune check-points are major cell surface inhibitory receptors that may also be involved in the chronicity of AIDs. Intracellular checkpoints contribute to limit the activation of inflammatory cells [66]. Thus a reduced expression of these molecules could prevent an efficient regulation of the immune cell activation. PD-1 SNPs have been associated with several AIDs including SLE and RA [67]. However, it is known that SNPs only rarely fully explain a phenotype and that other genetic variants should be concomitantly taken into account. This is illustrated by the PD-1 deficiency in mice, which results in the development of a lupus-like autoimmune disease or a cardiomyopathy depending on the genotype background [68, 69]. Immune checkpoint blockers (ICBs) have recently proven their remarkable efficiency against melanoma and non-small cell lung carcinoma, but they can induce immune-related adverse events. In addition to rashes, hepatitis or colitis, ICBs have been associated with the development or the exacerbation of MG in 22 cases [70]. This observation is all the more striking as the CTLA-4 gene is strongly associated with MG [71]. Interestingly, while anti-CTLA-4 treatments have been linked to the development of MG in some cases, humans bearing mutations of CTLA-4 develop a spectrum of autoimmune syndromes distinct from MG [72, 73]. Overall, this may be sufficient to precipitate autoimmunity in a predisposing ground.

4) As discussed above, Treg cell defects could be induced by the high level of inflammation. However, in a highly inflammatory environment, conventional T cells might be out of control, and resistant to suppression. If the Treg are unable to reduce the proliferation of Tconv cells, this will predictably result in a chronic pathological process.

Inflammation can also lead to a re-programming of Treg cells into pro-inflammatory Th17 cells (ex-Tregs), illustrating a vicious circle whereby inflammation (that can be induced by Th17 cells) leads to more inflammation. The pathogenic role of ex-Tregs has been demonstrated in various experimental autoimmune models including RA [74]. Conversely, resolution of inflammation is associated with the transdifferentiation of Th17 cells into Tregs [75]. However, since the defective Treg cells are unable to reduce the inflammation level, the autoimmune response is maintained at a high level, contributing to the chronicity of the disease

Conclusion

AIDs are typically complex diseases constituting major intellectual challenges for clinicians and biologists. The most advanced methods of genetic screening or immune screening seemed to be promising but did not thus far enable to fully understand the causes of AIDs. However, it is of utmost importance that we now better apprehend to which extend AIDs could be complex. Genetically, it is now clear that polygenic explanations did not fulfill expectations, and that more efforts are needed to understand how the interplay of environmental clues may have a phenotypic impact. Immunologically, it is of utmost importance to distinguish co-occurring dysregulations from potentially causing perturbations that may even have come back to normal states. Collectively, AIDs remain challenging to study, and interdisciplinary approaches will be more than ever essential to unravel the complexity and provide better and more personalized therapeutic approaches in the future.

Figure Legends

Figure 1: Autoimmune diseases: some common features

Cloud of terms frequently associated with Myasthenia Gravis, as well as with other autoimmune diseases. As the font of a term is bigger, as the number of AIDs associated with this term is higher.

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Term	Gene number	Fold enrichment	P value
Systemic Lupus Erythematosus	24	2.04	0.00071
Graves' Disease	11	2.84	0.0027
Crohn's Disease	14	2.22	0.0065
Multiple Sclerosis	30	1.58	0.0092
Celiac Disease	14	2.03	0.014
Primary Biliary Cirrhosis	7	2.87	0.025
Sarcoidosis	11	2.07	0.030
Systemic Sclerosis	10	2.18	0.030
Ulcerative Solitis	10	2.11	0.037
Sjogren's Syndrome	7	2.57	0.043

Table 1. AID genetic associations shared with EOMG.

A similarity was found in the category of "Genetic Association Database" that is a database of genetic association data from complex diseases and disorders. Diseases that show significant association with EOMG are listed. The "gene number" column indicates the number of genes common between MG and the other autoimmune diseases. The "fold enrichment » column indicates the ratio between two percentages: the percentage of genes of on term in the hit list (deregulated gene list in EOMG) divided by the percentage of genes of the same term in the list of genes of the category "AID genetic associations". As an example, the percentage of genes associated with systemic lupus erythematosus is around 2% in the whole list (category AID genetic associations) and 4% in the hit list. Thus the ratio between these two percentages is 2. In other words, when the ratio is 1, that indicates no enrichment. Here the fold-enrichments are between 1.58 to 2.87, showing a significant enrichment for the diseases shown in the table.

Adapted from ref 4 with Springer permission.

Gene-Id	Name	Number of diseases	Change in MG
929	CD14 molecule	3	UP
930	CD19 moleculeEO	3	UP
941	CD80 molecule	4	DOWN
6352	Chemokine (C-C motif) ligand 5	4	UP
356	Fas ligand (TNF superfamily, member 6)	4	UP
3383	Intercellular adhesion molecule 1	5	DOWN
3659	Interferon regulatory factor 1	4	DOWN
3553	Interleukin 1, beta	6	UP
3117	Major histocompatibility complex, class II, DQ alpha 1	6	UP
3119	Major histocompatibility complex, class II, DQ beta 1	9	UP
3122	Major histocompatibility complex, class II, DR alpha	5	UP
3123	Major histocompatibility complex, class II, DR beta 1	10	UP
4846	Nitric oxide synthase 3 (endothelial cell)	4	UP
4790	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	4	DOWN
26191	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	9	DOWN
7421	Vitamin D (1,25- dihydroxyvitamin D3) receptor	3	UP

Table 2. List of the genes dysregulated in EOMG as well as in other autoimmune diseases.

Many genes dysregulated in EOMG are also associated with other autoimmune diseases. Some of them could be associated with many autoimmune diseases such as MHC class II genes or PTPN22. The number of diseases presenting the dysregulated genes is indicated in the column "number of diseases". Whether the expression of the gene is upregulated or downregulated in MG is indicated in the last column. *Adapted from ref 4 with Springer permission*.

Disease	Target Organ	Organ with ectopic GCs
Myasthenia Gravis	**Muscle**	**Thymus**
Multiple Sclerosis	Brain	Brain
Rheumatoid Arthritis	Synovium	Synovium
Sjogren's Syndrome	Salivary glands	Salivary glands
Systemic Lupus Erythematosus	Systemic	Pre-germinal centers and plasma cell precursors in the blood
Autoimmune Thyroiditis	Thyroid	Thyroid
T1 Diabetes Mellitus	Pancreas	Pancreas (NOD mice)

Table 3: Germinal Centers in AIDs. Many AIDS display ectopic GCs in their target organs, while in MG, the ectopic GCs are found in the thymus and not in the target organ, the muscle.

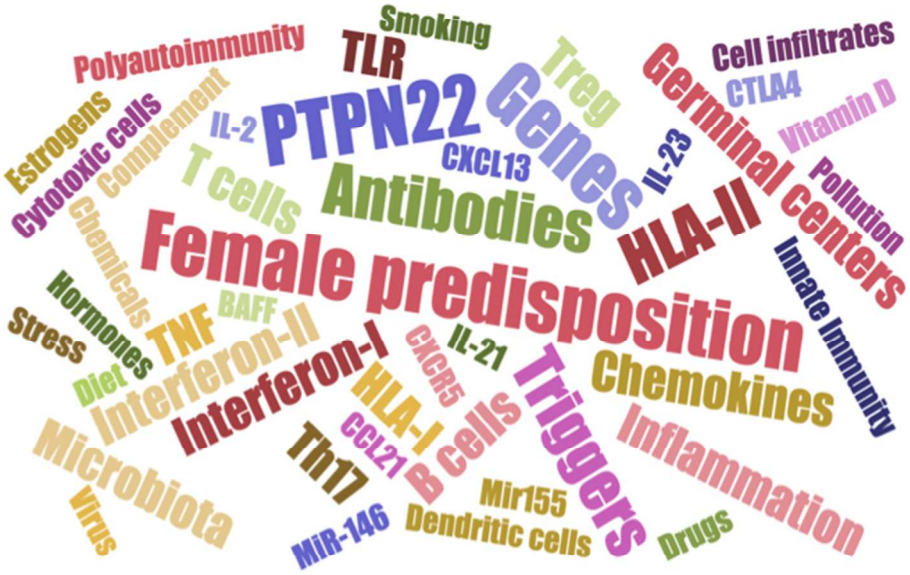

Figure 1

254x190mm (72 x 72 DPI)