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T Cells and Costimulatory Factors in Myasthenia Gravis

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To my family
“När jag blir stor ska jag gå på ett fnöre...”

ABSTRACT

The autoimmune disease myasthenia gravis (MG) is characterized by muscle weakness due to a loss of acetylcholine receptors (AChR) at the neuromuscular end plate. Most MG patients have pathogenic antibodies directed against the receptor. We provide further evidence that T cells are important for establishment and continuation of the disease. We have detected pathogenic antibodies capable of transferring disease to mice in healthy twin sisters as well as in their MG affected sisters in two monozygotic twin pairs discordant for MG. However, the healthy twin sisters did not demonstrate T cell responses against the AChR. A further support to the importance of T cells in maintaining the disease is shown in a successful treatment of an MG patient with antibodies targeting CD25. This molecule is expressed on activated cells. The levels of activated T cells, serum levels of IL-10 as well as the soluble costimulatory molecules sCD28, sCD80, sCD86 and sCD152 decreased, suggesting a normalization of an abnormally activated immune system.

Costimulatory molecules are important in the activation and inhibition of an immune response. We demonstrated reduced expression of the costimulatory molecule CD152 (cytotoxic T lymphocyte associated antigen 4, CTLA-4) in T cells from MG patients. CD152 is essential to inhibit an immune response, therefore the patients might have a reduced potential to down-regulate an ongoing immune reaction. We observed that the G allele at position +49 in coding sequence 1 of the CD152 gene was associated to increased immune activity, manifested as increased levels of IL-1β and $CD3^{+}CD28^{+}$ cells. MG patients with thymoma more frequently had the G/G genotype or the G allele, which could explain a more active immune response in patients with this genotype.

The costimulatory factors CD28, CD80, CD86 and CD152 also exist in soluble forms. The concentrations of sCD28, sCD80, sCD86 and sCD152, all of which recently have been shown to be increased in different diseases, were not increased in MG patients. However, in one of our studies we detected elevated levels of sCD152 in MG patients. The concentrations of sCD28, sCD80, sCD86 and sCD152 correlated to each other and to IL-6, IL-10 and IFN-γ. All four soluble costimulatory molecules correlated to sCD25 in healthy persons, while only sCD80 and sCD86 correlated to sCD25 in MG patients. In addition, we confirmed results by others demonstrating increased serum levels of sCD25 and sICAM-1.

We produced a recombinant form of the naturally occurring soluble costimulatory factor CD80. Recombinant sCD80 demonstrated capacity to interact with its natural ligands CD28 and CD152. It preferentially bound to activated cells. In addition, it displayed immunosuppressive properties, demonstrated by inhibition of T cell activation, inhibition of the mixed lymphocyte reaction and the ability to alter the cytokine secretion balance *in vitro*. The effect of sCD80 *in vivo* has to be clarified, but it is tempting to speculate about a potential future use of the soluble protein in treatment of diseases like MG.

In summary, we have provided further evidence that T cells are important in the initiation and maintenance of MG, and that the costimulatory factors could be involved in disease progression.

LIST OF PUBLICATIONS

The thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I. **Kakoulidou M**, Åhlberg R, Yi Q, Giscombe R, Pirskanen R, and Lefvert AK. The autoimmune T and B cell repertoires in monozygotic twins discordant for myasthenia gravis. *J Neuroimmunol*. 2004, 148 (1-2), pp 183-191

II. Wang XB, **Kakoulidou M**, Qiu Q, Giscombe R, Huang DR, Pirskanen R, and Lefvert AK. CDS1 and promoter single nucleotide polymorphisms of the CTLA-4 gene in human myasthenia gravis. *Genes Immun*. 2002, 3, pp 46-49

III. Wang XB, **Kakoulidou M**, Giscombe R, Qiu Q, Huang DR, Pirskanen R, and Lefvert AK. Abnormal expression of CTLA-4 by T cells from patients with myasthenia gravis: effect of an AT-rich gene sequence. *J Neuroimmunol*. 2002, 130 (1-2), pp 224-232

IV. **Kakoulidou M**, Wang XB, Zhao X, Pirskanen R, and Lefvert AK. Soluble costimulatory factors in relation to other markers of immune activation in patients with myasthenia gravis. *Manuscript*

V. **Kakoulidou M**, Giscombe R, Zhao X, Lefvert AK, and Wang XB. Human soluble CD80 is generated by alternative splicing. Recombinant soluble CD80 binds to CD28 and CD152 and inhibits T cell activation. *Submitted*

VI. **Kakoulidou M**, Pirskanen R, and Lefvert AK. Treatment of a patient with myasthenia gravis using antibodies against CD25. *Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

-/-	Deficient, knock-out
AChR	Acetylcholine receptor
APC	Antigen presenting cell
CD	Cluster of differentiation
cDNA	Complementary DNA
CDS	Coding sequence
ConA	Concanavalin A
CTLA-4	Cytotoxic T lymphocyte associated antigen-4 (CD152)
DNA	Deoxyribonucleic acid
EAMG	Experimental autoimmune myasthenia gravis
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot assay
FACS	Fluorescence-activated cell sorter / flow cytometer
HIV	Human immunodeficiency virus
HLA	Human histocompatibility leukocyte antigen
ICAM-1	Intercellular adhesion molecule-1 (CD54)
ICOS	Inducible costimulator
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
L	Ligand
LPS	Lipopolysaccharide
MG	Myasthenia gravis
MHC	Major histocompatibility complex
mRNA	Messenger RNA
MuSK	Muscle-specific receptor tyrosine kinase
NK cell	Natural killer cell
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHA	Phytohemagglutinin
PMA	Phorbol myristate acetate
R	Receptor
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
s	Soluble
SCID	Severe combined immunodeficiency
SLE	Systemic lupus erythematosus
T_H cell	T helper cell
TNF	Tumor necrosis factor

1 INTRODUCTION

1.1 MYASTHENIA GRAVIS

1.1.1 Background

Myasthenia gravis is an autoimmune disease characterized by muscle weakness. In 1672 Thomas Willis first described the disease, and about 300 years later the disease was suggested to be of autoimmune origin (1, 2). The weakness is due to a loss of AChRs at the neuromuscular endplate (3, 4). Upon propagation of an action potential, the neuron releases acetylcholine which binds to the receptor located on the muscle. The binding of acetylcholine to its receptor leads to contraction of the muscle. A reduction in the number of receptors impairs the muscle contraction, hence gives rise to the muscle weakness seen in the MG patients. The etiology of the disease is not known, although the main antigen, the AChR, to which the abnormal immune response is directed, is well characterized (reviewed in (5)).

All skeletal muscles can be affected. MG is classified as ocular (class 1), mild generalized (class 2a), severe generalized (class 2b), acute (3), late severe generalized (4) or remission (A). The prevalence of the disease is 14/100 000 and the sex ratio (females/males) is 1.7/1 in Stockholm, Sweden (6). MG can debut at any age, and there is a predominance of women between the ages 15 to 40 (6, 7). However, the incidence of late-onset MG is increasing, and in these patients the sex ratio is more balanced ((6) and reviewed in (8)).

1.1.2 Treatment

Autoimmune diseases involve an abnormal immune reaction against a self antigen. The suppression of an autoimmune disease, with the ability to preserve an alert immune response against harmful agents is a dream scenario. MG can not be cured today and there is only symptomatic treatment. However, progress has been made since the disease caused death in many cases before modern treatment started (9). The disease is nowadays treated with acetylcholine esterase inhibitors, which prolong the action of acetylcholine in the synapse. Patients can also get additional treatment consisting of immunosuppressive agents, as corticosteroids, methotrexate, azathioprine and cyclosporine, or immunomodulators such as high dose intravenous IgG (IVIg). Patients are generally thymectomized, although thymectomy is not recommended in patients with ocular MG, unless they show signs of thymoma. Plasmapheresis to treat MG was more common in the 1970s and 80s (personal communication with R Pirskanen).

Biological therapies for treatment of various diseases are emerging. Many patients with rheumatoid arthritis benefit from therapies directed against TNF-α (10, 11). A recent publication reported improvement in MG patients after treatment with recombinant soluble TNF receptor fusion protein (etanercept) (12). MG patients also benefit from elimination of B cells using anti-CD20 antibodies (13-16). Another antibody therapy, which we have used in a pilot study to treat an MG patient, is directed against the IL-2 receptor α-chain (also known as CD25). The antibody was first intended to prolong survival of grafts in transplantations (17, 18). Anti-CD25 therapy is also successful in

the treatment of psoriasis (19-22), chronic atopic dermatitis (23), erosive lichen planus (24), epidermolysis bullosa acquisita (25), aplastic anemia (26), uveitis (27, 28), steroid resistant ulcerative colitis (29), and multiple sclerosis (30, 31). In addition, rhesus monkeys with collagen induced arthritis benefit from treatment with daclizumab (Zenapax®) – the fully humanized anti-CD25 antibody – supporting a role for anti-CD25 therapy in an autoimmune disease like rheumatoid arthritis (32). Thus, more biological therapies are entering the therapeutical area, but the classical immunosuppressants are still in use. None of the therapies targets only those cells that are autoreactive, hence the treatments are not specific.

1.1.3 Genetic associations

Autoimmune diseases are complex disorders involving multiple genes and unknown environmental triggers. The fact that the familial incidence is higher compared to the incidence in the population in general, together with a higher concordance rate in monozygotic than dizygotic twins suggest a true genetic link for MG (33-36). Nevertheless, most autoimmune diseases including MG show a rather low concordance rate in monozygotic twins, who should be genetically "identical", pointing to the importance of environmental factors in disease development (37). Thus, the genetic background of a person determines the susceptibility to disease, but this alone is not enough to develop the disease.

Different autoimmune diseases often cluster within families, suggesting that common genetic factors exist for various autoimmune diseases. Indeed, one of the indicators that made Simpson suspect MG to be of autoimmune origin, was the clustering of the disease together with other known autoimmune diseases in families (1). The immune system assures protection to intruding pathogens, but an improper targeting of self structures gives rise to autoimmunity. The genes of the HLA-system are very good candidates as disease susceptibility genes because of their great impact on what the T cell will "see" and not "see" in a specific individual. The highly polymorphic HLA-molecules preferentially bind certain kinds of peptides, and therefore could significantly influence the immune response. MG is linked to different HLA-types, and the haplotype HLA-DR3 B8 A1 is associated to MG with thymic hyperplasia as well as to other autoimmune diseases (SLE, celiac disease, type I diabetes and autoimmune thyroiditis) (reviewed in (38)). Patients with thymoma show different HLA associations (reviewed in (38)). Even though there are some associations to specific HLA-haplotypes, the odds ratios are not very high. Thus, disease susceptibility involves other genetic factors. Each susceptibility locus might only have a minor contribution to the overall predisposition, and some could even protect from disease, leading to a complex genetic interplay.

Other genes of interest for development of MG are those important for immune cell function (the genes for IL-1β, IL-1 receptor antagonist, IL-10, TNF-α, CD152, Fc gamma receptors, the TCR, and GM allotypes of IgG), the β_2-adrenergic receptor, and obviously the autoantigen the AChR (39-56). The CD152 gene is very interesting in this context since polymorphisms in the gene have been associated to many other autoimmune diseases including Grave's disease, Hashimoto's thyroiditis, type I diabetes, multiple sclerosis, celiac disease, and vitiligo (reviewed in (57)). Furthermore,

the protein has a central role in turning off the immune response, making its association to autoimmune diseases more relevant (see below). The gene for CD152 is located at chromosome 2q33 in humans (58). It contains multiple polymorphisms. The most well-known are a C to T SNP in the promoter region at position -318 (59), an A to G mutation at position +49 in CDS1 (60), and an AT repeat at position +642 in the 3'-UTR (61) (Fig 1).

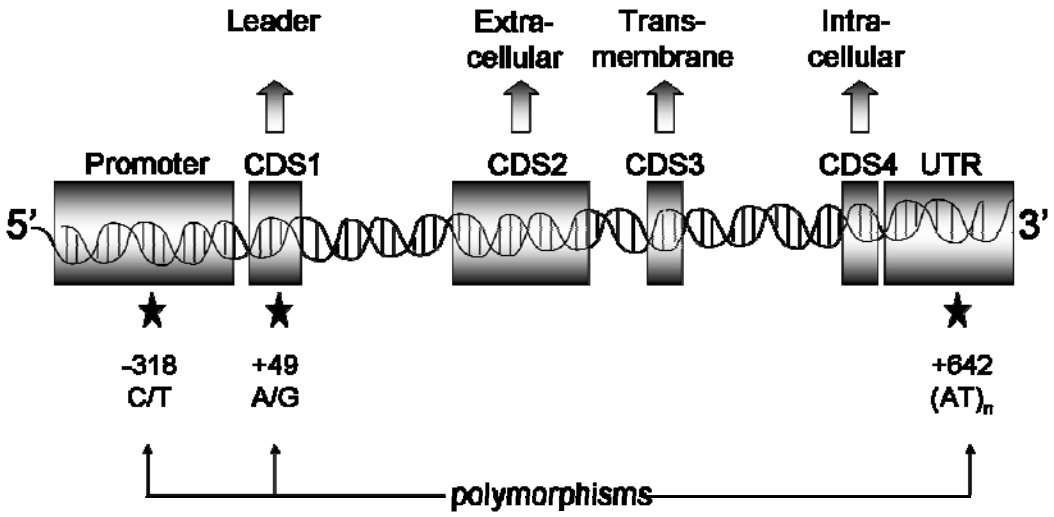

Fig 1. Schematic picture of the CD152 gene. The CD152 gene is located on chromosome 2q33 in human. The figure displays the best characterized polymorphisms. One polymorphism is present in the promoter region (-318 C/T), one CDS 1 at position +49 (A/G), and a microsatellite consisting of AT repeats is located in the 3'-UTR.

1.1.4 The thymus

The thymus is the place in which the T cell repertoire is shaped, where the positive and negative selection of thymocytes occurs. A majority of MG patients have thymic abnormalities (62). Younger females often have thymic hyperplasia, and thymoma occurs in about 15% of the patients, mostly in older patients (6, 7, 62, 63). Patients with thymoma generally have a more severe and aggressive disease (7, 64). The importance of the thymus for disease development was illustrated by the induction of disease after transfer of thymus tissue from MG patients to SCID mice (65), and the fact that many patients benefit from thymectomy (66, 67). The hyperplastic thymus possesses all the ingredients needed to start an immune response against the autoantigen; autoreactive T_H cells, APCs, the autoantigen the AChR, as well as antibody secreting plasma cells (68-73). However, the cells reactive to the AChR might also have entered the thymus after a sensitization elsewhere (72). Since antibodies against citric acid extract of human skeletal muscle stained both skeletal muscle and epithelial cells in thymoma tissue, it was hypothesized that thymoma patients could develop the disease because of a cross-reactivity between a thymoma related antigen and skeletal muscle (74). Another model described a scenario where the thymoma produces non-tolerant naïve T cells that are exported and activated in the periphery (reviewed in (75)).

1.1.5 B cells

B cells become antibody secreting plasma cells upon activation. MG is considered to be a B cell driven disease since about 90% of the patients have autoantibodies against the AChR (76, 77), and these antibodies play a major role for the reduced receptor function at the endplate. The antibodies have the capability to transfer disease to animals (78, 79), and injection of AChR also causes disease with production of anti-AChR antibodies in animal models (80, 81). The antibodies can function via 1) inhibition of the binding of acetylcholine to the receptor (82), 2) increased internalization of the receptors (83, 84) and/or 3) activation of the complement cascade leading to destroyed endplates (85). However, about 10% of the patients with MG do not have antibodies against the AChR, but still show the symptoms of the disease, and their Ig fractions and plasma can transfer the disease to mice (86, 87). The presence of ocular symptoms only is more common in these "seronegative" patients, and the patients may be different from MG with anti-AChR antibodies (reviewed in (88, 89)).

Other autoantibodies have also been demonstrated in MG patients, for example antibodies against the muscle antigens titin, MuSK (muscle-specific receptor tyrosine kinase), the ryanodine receptor, actin, actinin, actomyosin, myosin and the β_1- and β_2-adrenergic receptors (90-96).

Antibodies consist of constant and variable regions. The variable domain of the antibody (the idiotype) has been proposed to constitute an antigen in itself, able to trigger the production of anti-idiotypic antibodies. In the same way, anti-anti-idiotypic antibodies can arise, generating a network of antibodies able to react with each other. Jerne anticipated the theory about this "idiotypic network" in the 1970s (97). The anti-idiotypic antibody could represent a mirror image of the true antigen, and anti-anti-idiotypic antibodies may have the capacity to react with the antigen. The anti-idiotypic antibodies may either down-regulate the immune response, or trigger the production of pathogenic antibodies (97). The anti-idiotypic antibodies exist in MG patients, and they can be induced in experimental animals, suggesting that the idiotypic network is operating in MG patients (98-103) (Fig 2). An anti-idiotypic antibody was able to induce production of anti-AChR antibodies, and could thus lead to development of disease (104). The levels of the anti-idiotypic antibodies showed an inverse relationship with anti-AChR IgG antibodies, suggesting that they are either regulatory or involved in expansion of pathogenic cell clones (105). In addition, since healthy relative to MG patients have the anti-idiotypic antibodies, the antibodies have a potential regulatory role (106).

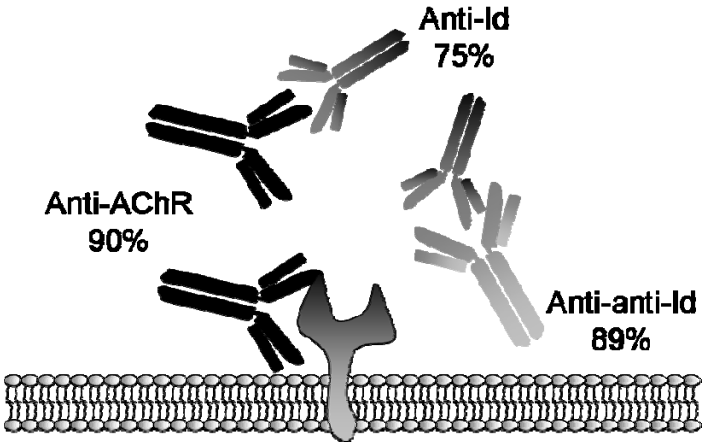

Fig 2. The idiotypic network in myasthenia gravis. Approximately 90% of MG patients have anti-acetylcholine receptor (AChR) antibodies (76, 107). 75% of the patients were reported to have anti-idiotypic (id) antibodies, directed against the variable region, and 89% had anti-anti-idiotypic antibodies (103).

The importance of B cells in MG is further illustrated by the findings that mice lacking B cells did not develop EAMG upon immunization with AChR, and that patients treated with antibodies depleting the B cells improved in their disease (13-16, 108, 109).

1.1.6 T cells

B cells need help from T cells to become plasma cells and secrete antibodies. This is true for most antigens, including the AChR, as shown in animal studies (110, 111). The T cell mediated activation of B cells is predominantly via $CD4^+$ T_H cells, both via cell to cell interactions and via cytokines. Therefore, the T cells have been a major focus in the study of MG since the AChR specific T cells were found in MG patients (112-115). MG can not be induced in thymectomized rats, and the disease can be transferred by lymphocytes (111). Transfer of cells depleted of $CD4^+$ or $CD8^+$ cells from MG patients to SCID mice demonstrated the need of $CD4^+$ cells for development of the disease (116), while others showed that both $CD4^+$ and $CD8^+$ cells are important for the disease to develop in mice (117). The fact that T cells are important to maintain the disease was demonstrated in a successful treatment study of a patient receiving antibodies against CD4 (118). Furthermore, genetic studies support the significance of factors central to T cell function in MG, since polymorphisms in the genes for IL-1β, IL-1 receptor antagonist, IL-10, CD152, and TNF-α were linked to MG, or subgroups of MG (39-43). Thus, all of the above findings support a significant role for T cells in the pathogenesis of MG.

There are several subgroups within the $CD4^+$ T_H cell population. IL-12 can induce T_H1 cells, which secrete IL-2, IFN-γ and TNF-β, leading to cell mediated immunity. IL-4 induces T_H2 cells, which secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, leading to humoral immunity. Mouse studies pointed to the importance of IFN-γ and IL-12 in the development of EAMG (119, 120). Rats on the other hand did not require IFN-γ to

develop disease (121). However, blockade of IL-18 or CD40L in rats, as well as oral tolerance to a peptide of human AChR, down-regulated EAMG leading to suppression of T_H1 cytokines (122-124). The T_H2 cytokine IL-4 was not required for disease induction, and the authors of the article suggested that T_H2 cells might even protect from disease (125, 126). The importance of IL-10 in the disease is obscure since this cytokine has multiple functions and can act both as a pro-inflammatory and an anti-inflammatory cytokine. It seems to aggravate MG since mice transgenic for IL-10 exhibited accelerated EAMG, recombinant IL-10 exacerbated disease, IL-$10^{-/-}$ mice demonstrated reduced disease symptoms, and MG patients had increased levels of AChR specific IL-10 secreting cells (127-130). Both T_H1 and T_H2 AChR reactive cells were detected in MG patients (131). Although, it is not clear what role the different subgroups play in human MG, since the contributions of T_H1 and T_H2 cytokines to the disease may vary in different species.

In addition to the conventional $CD4^+$ T_H cells and $CD8^+$ cytotoxic cells, there are regulatory subsets of T cells. The thymus derived $CD4^+CD25^+$ naturally occurring regulatory T cells, characterized by their ability to suppress other cells, were first demonstrated in mice followed by detection in humans and are since then a hot area of investigation (132-135). These cells are important to prevent autoimmunity since mice deficient of CD25 develop autoimmune diseases (136). The $CD4^+CD25^+$ regulatory T cells have also been explored in MG patients. The thymus in thymoma patients contained reduced numbers of $CD4^+CD25^+$ regulatory cells (137, 138), while another group has reported no difference in their number but an impaired function (139). Reports also diverge regarding the number of $CD4^+CD25^+$ regulatory T cells in the peripheral blood – some demonstrated no difference between healthy persons and patients (including different subgroups) (137, 139, 140). Another study showed decreased numbers of the cell type in thymoma patients (138). Patients with stable MG even had increased levels of $CD4^+CD25^+$ cells compared to "uncontrolled" MG patients and healthy persons (141). Thymectomized patients also exhibited increased levels of the regulatory cells as compared to non-thymectomized patients and healthy controls in the same study (141). In addition, treatment may influence the number of these cells since MG patients without treatment had decreased numbers of the $CD4^+CD25^+$ cells compared to healthy persons, and patients with immunosuppressive treatment had increased numbers of the regulatory cells compared to patients without treatment (142). There was also a tendency to similar findings in thymus tissue from the patients (142). The transcription factor FoxP3 is important for the function of the regulatory T cells, and might be a specific molecular marker for this cell type (reviewed in (143)). Thymocytes from MG patients in general and MG patients with thymoma, as well as PBMC from thymoma patients, contained reduced levels of this transcription factor (138, 139). Thus, the function of the $CD4^+CD25^+$ regulatory T cells seems to be impaired in aberrant thymi of MG patients, which in some cases is reflected in the peripheral blood.

1.2 COSTIMULATION IN THE ACTIVATION AND INHIBITION OF T CELLS

1.2.1 Activation and inhibition by CD28 and CD152

In 1970, Bretscher and Cohn proposed the theory that T cells need two signals to become activated (144). The first signal provides specificity, assuring stimulation only of those T cells specific for the antigen. MHC with a bound peptide interacting with the TCR delivers this signal. The second signal is transmitted via costimulatory molecules. CD28 is probably the most important costimulatory molecule for the start of an immune response, while CD152 (CTLA-4) is important for the down-regulation of the response. The cell activation via CD28 leads to up-regulation of survival genes (Bcl-x_L) (145, 146), enhanced production and stabilization of IL-2 and other cytokine mRNAs (147, 148), as well as cell cycle progression (149, 150). CD152 ligation on the other hand inhibits cell cycle progression and IL-2 production, as well as blocks the expression of CD25 (151-153). T cells express CD28 and CD152, which both interact with CD80 and CD86 on the APCs. T cells and APCs constitutively express CD28 and CD86, respectively, while expression of CD152 and CD80 is induced upon activation (154-162) (Fig 3). However, T cells can also express CD80 and CD86 upon activation, and acquisition of CD80 from APCs occurs in both mouse and human T cells (160, 163-166). The functional significance of the expression of CD80 and CD86 on T cells is not clear, but the cells have the possibility to act as APCs themselves providing costimulation to other T cells (163, 164). CD80 is a more potent costimulator than CD86, and the affinity to CD152 is higher compared to CD28 (167-170).

CD152 is expressed on the surface of activated T cells, but most of the protein exists in intracellular stores, wherefrom it cycles to the surface (171, 172). Recently, a form of CD152 (CD152li) lacking the CD80/CD86 binding domain was demonstrated in mice (173). Even though it lacked the ligand binding part, it had the ability to deliver an inhibitory signal to the T cell. Similarly, human T cells express a splice variant of CD28 (CD28i) lacking most of the extracellular part (174). This molecule could amplify the costimulatory signal via CD28 (174). Thus, not only are the receptor and ligand interactions complicated, splice variants of the different molecules add more complexity to the system.

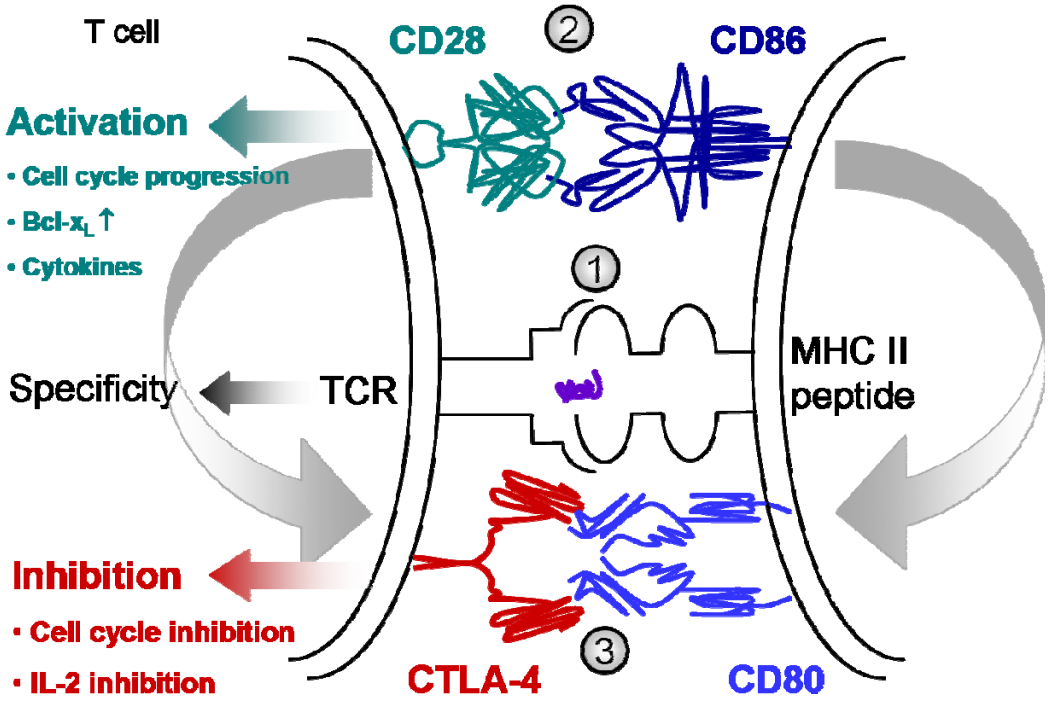

Fig 3. Activation and inhibition of T cells by CD28 and CD152. CD28 and CD152 on the T cell can both interact with CD80 and CD86 on the APC. For simplicity, the figure only illustrates one of the two possible interactions for each molecule. 1) The TCR on the T cell interacts with MHC with a bound peptide on the APC. This allows only those T cells specific for the peptide to become activated. 2) CD86 (or CD80) on the APC interacts with CD28 on the T cell, delivering the second signal. This interaction leads to activation of the T cell. 3) Later on in the immune response, up-regulation of CD80 on the APC and CD152 on the T cell takes place. Ligation of CD152 (via CD80 or CD86) leads to inhibition of the T cell response.

Mice deficient in specific genes can give a lot of information about the contribution of a gene to a specific phenotype. However, different genetic backgrounds of mice can influence the phenotype. Mice lacking the CD152 gene demonstrate the importance of CD152 for the immune system to function properly. The $CD152^{-/-}$ mice showed massive lymphoproliferation, and died within a few weeks after birth (175). $CD4^+$ T cells induced this phenotype, as deletion of these cells prevented lymphoproliferation (176). The $CD152^{-/-}$ mice could be rescued by addition of CTLA4-Ig (a chimeric protein consisting of the extracellular part of CD152 and the Fc part of IgG) (177). On the other hand, normal B and T cell development does not require CD28, as demonstrated in the $CD28^{-/-}$ mouse (178, 179). Immune responses occurred in these mice, but they showed reduced T cell proliferation, as well as reduced T_H cell activity and Ig switching. However, they had a normal cytotoxic T cell response against virus. Mice deficient in both CD80 and CD86 demonstrated that these two molecules are central for switching to IgG, which is dependent on T cell help (180). $CD86^{-/-}$ mice had differences in antibody switching compared to wild type mice depending on the immunization route (180). The effect in $CD80^{-/-}$ mice was not that pronounced. CD80 and CD86 had overlapping effects on class switching and germinal center formation,

with CD86 having a greater effect (180). Thus, the costimulatory molecules are very important for a normal immune response to operate in a proper and efficient way, and the different "knock-out" models can be used to clarify the significance of the specific molecules as well as to elucidate the roles of other costimulatory pathways. Moreover, the CD152 gene might be a common susceptibility gene for autoimmunity (57, 181). Polymorphisms in the gene affecting the expression of the protein derivatives can have devastating effects on the outcome of an immune response, since mice lacking the molecule die at an early age (175).

1.2.2 Soluble costimulatory factors and their function

The finding of soluble forms of CD28, CD80, CD86 and CD152 in humans adds more complexity to the network of costimulation (182-185). Reports of increased levels of these proteins in patients compared to controls have appeared in recent years (summarized in Table 1). Asthmatic children and patients with primary Sjögren's syndrome, SLE and systemic sclerosis showed increased levels of sCD28 (184, 186, 187); while children and adults with asthma, patients with autoimmune thyroid disease, SLE and diffuse cutaneous systemic sclerosis had elevated levels of sCD152 (186-191). Patients with hematological malignancies and SLE demonstrated increased levels of CD80 and sCD86 (183, 186, 192). Adults with acute asthma and asthmatic children also had increased levels of sCD86 and sCD80, respectively (187, 193). Thus, the presence of these soluble costimulatory molecules may be used as markers of immune activity in different patient groups, but their functional significance has yet to be proven (see below).

Table 1. The levels of the soluble costimulatory factors sCD28, sCD152, sCD80 and sCD86 are increased in various disease states. For simplicity, the group called "asthma" includes both children and adults with asthma. The respective references are given within brackets.

	sCD28	sCD152	sCD80	sCD86
Asthma	✓* (187)	✓*,▽ (187, 191)	✓ * (187)	✓ ▽ (193)
SLE	✓ (184, 186)	✓ (186, 189)	✓ (186)	✓ (186)
Hematological malignancies			✓ (183)	✓ (192)
Systemic sclerosis	✓ (184)			
Diffuse cutaneous systemic sclerosis		✓ (190)		
Sjögren's syndrome	✓ (184)			
Autoimmune thyroid disease		✓ (188)		

* Increased in children with asthma

▽ Increased in adults with asthma

Reports on the function of the soluble costimulatory factors are not consistent. CD152Ig, a chimeric protein consisting of the extracellular part of CD152 and the Fc domain of IgG, down-modulates immune responses, shown *in vitro* as well as in animal models, and recently even in rheumatoid arthritis patients in human studies (169, 194,

195). Soluble CD152 lacking the transmembrane domain also demonstrated inhibitory activity (185). The reports of the function of soluble forms of CD28, CD80 and CD86 are inconsistent. *In vitro* as well as mouse models showed augmented anti-cancer responses by CD80Ig and CD86Ig (196-198). On the other hand, co-delivery of a plasmid coding for the extracellular part of CD86 inhibited the immune response against DNA-vaccines in mice (199). Stimulation of T cells with CD80Ig or the extracellular part of CD80 in immobilized form led to proliferation, while the protein in soluble form did not (168, 199-202). A non-glycosylated form of extracellular CD80 even suppressed T cell proliferation, but in another study a similar protein gave the opposite result (200, 203). An inhibitory effect was also observed with porcine soluble CD80 containing a histidine-tag (204). Soluble CD86 (lacking the transmembrane region) exhibited a stimulatory role in T cell activation *in vitro* (182). Soluble CD28, produced in COS cells as a protein lacking the transmembrane region, inhibited T cell proliferation (184). The effects of the chimeric Ig-forms of the two T cell associated molecules CD28 and CD152 in dendritic cells have also been investigated. CD28Ig stimulated dendritic cells to secrete IL-6 and to become "activated" – having the opposite effect as compared to CD152Ig on this cell type (205, 206). In this system, CD28Ig enhanced an anti-cancer response in mice (206). Hence, the function of the soluble costimulatory factors is not completely elucidated, and further studies are needed to confirm if the chimeric forms of the proteins possess the same functional characteristics as the natural soluble forms. It is also important to confirm the true effect of the naturally occurring soluble costimulatory factors *in vivo*.

1.2.3 Other costimulatory pathways

Other costimulatory pathways can operate between the T cells and the APCs, for example positive signals can be mediated by interactions via ICAM-1/LFA-1 (lymphocyte function-associated antigen), ICOS/ICOS-L, OX40/OX40L, CD40/CD40L and 4-1BB (CD137)/4-1BBL, while negative signals can be supplied by PD-1 (programmed death-1) interacting with PD-L1 or PD-L2, and BTLA (B and T lymphocyte attenuator) interacting with B7H-4 (reviewed in (207, 208)). Thus, the costimulatory network is redundant assuring for activation and inhibition of the T cells, although CD152 is essential to prevent autoimmunity (175).

1.2.4 Costimulatory molecules in myasthenia gravis

The fact that $CD80^{-/-}$, $CD80^{-/-}/CD86^{-/-}$, $ICOS^{-/-}$ and $CD40L^{-/-}$ mice do not develop EAMG, and $CD28^{-/-}$ mice are less susceptible to disease induction show the importance of the costimulatory pathway in MG (209-211). Antibodies to CD152 aggravated EAMG in mice (212), and CTLA4-Ig treatment of rats with EAMG improved the disease (213). The expression of the membrane bound costimulatory molecules in MG patients was different from that in healthy persons. Patients had decreased levels of $CD8^{+}CD28^{+}$ cells, and increased levels of $CD4^{+}$, $CD8^{+}$ and $CD14^{+}$ cells expressing CD80 and CD86 (214). Together the results suggest that the costimulatory molecules are likely to be involved in the improper immune reactivity in MG patients. Manipulation with their expression or providing them as drugs could affect the disease outcome.

2 AIM OF THE STUDY

The study was intended to investigate the role of T cells and costimulatory molecules in myasthenia, with particular emphasis on CD152. The following questions were addressed:

- Is there any difference in the T cell or B cell responses to disease related antigens in two monozygotic twin pairs discordant for MG?
- Are there associations between MG and the most well studied CD152 gene polymorphisms?
- Is the expression of CD152 abnormal in MG patients?
- Do MG patients have altered serum levels of the soluble costimulatory factors sCD28, sCD80, sCD86 and sCD152, and do these proteins correlate to other known markers of immune activation?
- What is the function of soluble CD80?
- Can MG patients benefit from CD25 targeting?

3 METHODOLOGICAL CONSIDERATIONS

3.1 DNA

3.1.1 Extraction of DNA

DNA was extracted from whole blood using the chloroform/phenol method or the salting out method. The latter method is to prefer since it avoids the use of harmful chemicals.

3.1.2 Detection of DNA variants

An SNP can give rise to a change in a restriction enzyme recognition site, or such a site can be introduced into a sequence with the use of primers. A specific base in the SNP will permit digestion of the PCR-amplified product by a restriction enzyme, hence discrimination of different alleles can be performed after visualization of the bands on an agarose gel. An advantage with this method called “restriction fragment length polymorphism” is its simplicity and that it is not too time consuming. Sequencing is another method to study genomic differences. We used sequencing to calculate the length of a microsatellite in the CD152 gene and to obtain the sequence for splice variants of CD80.

3.2 RNA

3.2.1 Purification of RNA

To prepare RNA we used Ultraspec II™, which is based on guanidine and urea as denaturing agents, chloroform to extract RNA and a specific RNA binding resin to purify the RNA. In earlier studies we used RNAzol B. A disadvantage with the two methods is the use of harmful chemicals as chloroform and phenol.

3.2.2 RT-PCR and real time PCR

RT-PCR was used to qualitatively detect RNA in different cell types. Real time PCR is another method to measure mRNA/cDNA amounts. Real time PCR is sensitive and gives a quantitative aspect of the presence of different mRNAs. A fluorescently labelled probe detaches from the target sequence as elongation occurs. The more cycles of amplification, the more fluorescence will be released and detected. The major difference between RT-PCR and real time PCR is that with real time PCR one can measure the amount of mRNA during the exponential synthesis. An alternative to the methods is Northern blot, where the PCR amplification step is eliminated and detection of different RNAs is done using radioactively labelled probes. However, with this method one needs more cells to start with, and the radioactive component is a drawback.

3.3 PROTEIN

3.3.1 Protein expression and purification

We used the *Escherichia coli* expression system to express recombinant soluble CD80 containing a histidine-tag. The *E. Coli* system is fast and usually gives a high yield of the protein of interest, but it does not give the same post-translational modifications of the protein as mammalian cells. We purified the protein taking advantage of the high affinity of nickel ions for the histidine tags. To use the soluble recombinant CD80 in biological assays, we refolded the protein in order to obtain a more native form of it. Since there is no "gold standard" how to do this – different proteins need different buffers and agents to obtain a biological functional conformation – refolding of proteins can be a tedious work.

3.3.2 Detection of proteins

Proteins can be detected in different ways, some more sensitive than others. I will here briefly summarize the protein detection methods I have used.

3.3.2.1 Coomassie staining of gels

After gel electrophoresis gels can be stained with Coomassie staining. This method does not discriminate between proteins – all proteins are labelled. The method can be used to see the purity of a sample. We have used the method to visualize protein bands that were cut out and further analyzed using mass spectrometry.

3.3.2.2 Western blotting

Western blotting was used to detect proteins after gel electrophoresis. The method relies on the equilibrium reaction between antibodies specific for certain epitopes and the protein of interest. The specificity of the antibody as well as the affinity of it determines the successfulness of the method. Some antibodies might be cross-reactive with epitopes present in other proteins. False positive results are therefore a drawback with the method.

3.3.2.3 Immunoadsorption

Human serum has very high protein content. If a protein of interest is present in a very low concentration, its detection can be blurred by the high concentrations of other proteins as antibodies or albumin. In this study we used immunoadsorption to concentrate soluble CD80 from serum samples.

3.3.2.4 RIA

RIA is a technique that is based on the binding of antibodies to a specific radiolabelled protein**.** The method is widely used to detect antibodies against the AChR. A disadvantage with the method is the use of radioactively labelled iodine. An ELISA based on a cell line expressing AChR is an alternative method (215). However, the use of human muscle extract instead of receptor from a cell line is preferred since the cell line does not express all forms of the receptor (216). The RIA is also more sensitive.

3.3.2.5 ELISA

ELISA is based on a non-competitive antibody pair binding to a protein of interest. In this study, the “sandwich method” was used to detect different soluble proteins, mostly in serum but also from cell culture supernatants. The technique is not too sensitive; it can be used to discriminate positive samples from negative, but absolute concentrations should be regarded with caution.

3.3.2.6 ELISPOT

ELISPOT is like the ELISA based on pairs of antibodies with specificity for a given protein. It differs from the ELISA in the sense that a cell suspension is cultured in antibody coated wells. If the cells secrete the protein of interest (eg a cytokine or an antibody) the protein will bind to the antibodies beneath the cell, giving rise to a “spot”. The number of cells secreting a specific protein can then be counted in a microscope. A disadvantage with the method is the subjective part when the researcher has to decide what should be regarded as a spot or not, since artefacts are quite common and can interfere with the analysis.

3.4 CELLS

3.4.1 Thymidine incorporation

A widely used method to study proliferation is based on the incorporation of tritiated thymidine into growing cells. The incorporation of the radioactively labelled DNA base analogue is measured with a beta-counter. We used this method to study proliferation of PBMCs in cell stimulation assays as well as in the mixed lymphocyte reaction. Alternative methods based on for example the incorporation of BrdU (5-bromo-2'-deoxyuridine) or tetrazolium compounds into cells with colorimetric or chemiluminescent detection circumvent the use of radioactively labelled material.

3.4.2 Flow cytometry

Flow cytometry using a FACS is based on the specific binding of fluorescently labelled antibodies or chemicals to cells. Positive cells are discriminated from negative cells using a flow cytometer and analysis software. The technique is very fast compared to the cumbersome work of detecting and numbering cells in a microscope. It is used to detect proteins expressed at the surface as well as intracellularly after permeabilization of cells. The DNA content can also be detected, allowing one to compare apoptotic, necrotic and viable cells.

3.5 MOUSE

Passive transfer of human antibodies to mice can reveal if antibodies are pathogenic or not. C57BL/6 mice, a strain that is susceptible to EAMG can be injected with Ig fractions from persons. If present, pathogenic antibodies against the AChR will decrease the number of receptors at the endplates in the mice, and will also transfer the characteristic symptoms of the disease – the muscle weakness.

3.6 SUBJECTS

All MG patients included in the study were from the Stockholm area, Sweden. Samples were collected at the Myasthenia Gravis Centrum at the department of Neurology, Karolinska hospital. An established bank with DNA and sera from MG patients has been collected during many years. This bank was used for the genetic studies and for studying soluble factors in serum.

The healthy controls were from the Stockholm area, either healthy blood donors or persons recruited from the neighbouring laboratories.

3.7 STATISTICAL METHODS

Statistics were calculated with the InStat program (paper II and III) or GraphPad Prism version 4.00 for Windows (paper IV and V). The student's T-test (parametric) and the Mann Whitney test (non-parametric) were used to compare differences between groups. For non-parametric correlations we used Spearman correlations (paper IV). Linear regression was used to correlate AChR-antibodies to sCD152 levels (paper III), and to correlate humoral factors to age (paper IV). For genetic comparisons we used Fisher's exact test and the Chi-square test.

4 RESULTS AND DISCUSSION

4.1 ANTIBODIES IN MONOZYGOTIC TWINS DISCORDANT FOR MG (PAPER I)

MG, being one of the best characterized autoimmune diseases with a defined autoantigen, is often associated with antibodies against the autoantigen, the AChR (76, 77). Patients can also have anti-idiotypic antibodies (100-103), although the exact function of these antibodies is not clear. However, only having the antibodies against the disease associated antigens is not enough to develop the disease. When we compared two MZ twin pairs who had been discordant for MG for many years, we found that autoantibodies against the disease related antigens existed in the healthy twin sisters (Fig 1, paper I). In this regard they resembled a myasthenic population since healthy people do not normally have these autoantibodies (76). The antibodies have been demonstrated in healthy relatives to MG patients, suggesting that the presence of the antibodies does not have to lead to disease (106). The antibodies from the healthy twin sisters could transfer disease to mice, indicating that the antibodies are pathogenic and not just directed against an irrelevant region of the receptor. Moreover, EBV-transformation generated antibody producing cells of similar frequencies within the twin pairs, further supporting the fact that the antibody producing cells were present in the healthy twin sisters as well (Fig 2, paper I). EBV transforms cells that express CR2 (the C3d receptor also known as CD21) (217). This marker is not present on proliferating cells (218, 219) or on plasma cells (220). Thus, the healthy twin sisters as well as the myasthenic sisters had 1) cells that were producing the pathogenic antibodies (Fig 1, paper I) as well as 2) cells that could be induced to produce the antibodies (Fig 2, paper I). Still, the healthy twin sisters had not developed MG. It is unlikely that they will develop the disease, since both twin pairs have been discordant for the disease for more than 30 years. Other possible factors that could be involved in causing the discordance might be differences in the autoantigen, the end plate structure or the complement within the pairs. The complement system is an appealing contributor to MG since mice depleted in C3, and mice lacking C3, C4 or C5 develop less EAMG (79, 221, 222), while mice lacking decay-accelerating factor (DAF or CD55) are more prone to develop EAMG (223). Molecular mimicry due to cross-reactivity between an infectious agent and the AChR might also play a role. Alternatively, epigenetic factors, mosaicism, chimerism or haploinsufficiency may contribute to disease discordance in twins (224-226). The differences seen between the twin sisters could of course also be due to somatic mutations. Probably other factors than the genetic background show their contribution to disease development.

4.2 T CELLS ARE NEEDED TO START AND MAINTAIN MG (PAPER I AND VI)

T cells provide help to B cells to start an antibody response against an antigen like the AChR, and to perform class switching (110, 111). T cells are in this regard essential in the development of MG, and AChR specific T cells exist in MG patients (112-115). In addition, the T cells are important in disease development as shown in transfer experiments in animal studies (116, 117). Furthermore, depletion of $CD4^+$ cells in a

patient with MG had a beneficial effect on the disease outcome (118), supporting the fact that T cells are important for the progression and maintenance of the disease. In the twin study we could see that the T cell responses against the disease related antigens differed in the patients and their healthy sisters. The healthy twin sisters did not have any T cell response against the main autoantigen, the AChR (Fig 3, Table 1 and 2, paper I). In this regard they resembled a healthy population more than a myasthenic one. The T cells must have been present in the healthy twin sisters at some point in life, since these persons had the antibodies to the AChR. This suggests that the T cells specific for the AChR in the healthy sisters might have been 1) deleted, 2) rendered anergic or 3) suppressed.

Another aspect of the importance of T cells to maintain the disease comes from our pilot study where a patient with severe MG was repeatedly treated with antibodies against CD25 during 9 months. The $CD4^+$ activated cells decreased and the naïve cells increased (Fig 2 A-D, paper VI), suggesting a normalization of the immune response by deletion of activated T cells and/or recruitment of naïve cells. The $CD8^+$ cells demonstrated similar changes (Fig 3 A-D, paper VI). We detected a decrease in the serum levels of the soluble costimulatory factors sCD28, sCD80, sCD86 and sCD152, as well as a decrease in the amount of IL-10 (Fig 6 A-D and G, paper VI). Different studies point to a pathogenic role of IL-10 in MG (127-130). The decrease in IL-10 in the patient could thus indicate an improvement in disease. In addition, the decrease in the levels of the soluble costimulatory factors sCD28, sCD80, sCD86 and sCD152 suggests an improvement in the disease, as these markers are increased in various disease states, supporting a role for them as markers of immune activation (183, 184, 186-193). The patient felt better during the treatment period, and therefore corticosteroid therapy was discontinued (Fig 1 C, paper VI). The antibody therapy is directed against the $CD25^+$ cells, and we were not able to detect this cell subset with flow cytometry during the treatment phase. The cells could have been deleted. Previous studies in transplantations suggest that the antibody is only hindering the receptor from interacting with IL-2 (227), but also that it in addition to the previous mentioned masking of CD25 induces deletion of cells expressing the molecule as well as gives rise to shedding of CD25 (18). Even if the $CD25^+$ cells are deleted, CD25 is masked and/or stripped, the function of the activated cells will be suppressed, leading to disease amelioration. Collectively all these results suggest an improvement in disease at the immune cell level with the administration of anti-CD25 antibodies. However, the treatment has a potential risk since it can interfere with the $CD4^+CD25^+$ regulatory T cells, which are important for preventing autoimmunity. This potential risk might be more pronounced in a healthy person, who is not suffering from an autoimmune disease. Patients with this kind of diseases have an improper abnormal activation of cells. In addition, only eliminating the $CD4^+CD25^+$ cells in mice did not induce autoimmunity (228). Long-term treatment of patients with uveitis using a similar antibody was well-tolerated (28), supporting the use of anti-CD25 antibodies to treat autoimmune diseases. Nevertheless, the patient in our study developed side effects due to the treatment, hence the therapy was ended.

Together the results imply that T cells play a major role in the induction of the disease, as shown in the twin study, as well as in maintaining the disease, seen in the treatment of a patient with anti-CD25 antibodies.

4.3 CD152 POLYMORPHISMS IN MG (PAPER II AND III)

Both genetic background and environment are important for an autoimmune disease to start. Multiple genetic predisposing and preventing factors provide the setting for susceptibility to autoimmunity, and with the interplay of environmental triggers the disease will take form. The small contribution of each locus leads to the need of large study groups, which is a limitation for most researchers. MG is linked to the HLA-gene, the AChR gene, the β_2-adrenergic receptor gene and genes important for the immune system (38-47). The CD152 gene is one of them. Since the protein plays a vital role in the down-regulation of the immune response, genetic aberrancies in the gene may cause general susceptibility for autoimmunity (57, 181, 229). The CD152 gene contains numerous polymorphic sites. Many autoimmune diseases are linked to the most studied polymorphisms: the -318 in the promoter region (C to T mutation), the A to G mutation at +49 in CDS1 and the microsatellite in the 3'-UTR ((59-61) and reviewed in (57, 181)). However, it is not known if they really are involved in causing disease or merely just linked to other truly disease causing alleles. Longer AT repeats are associated to MG with thymoma (40), and we have shown that the length of the AT repeat influences the stability of the mRNA – the longer the repeat the more unstable the mRNA (Fig 4 and 5, paper III). There are associations between longer AT repeats in the 3'-UTR of the CD152 gene and increased levels of sCD25, plus an association to a greater immune response upon activation with anti-CD3 and anti-CD28 in MG patients (230). A recent publication supported this finding, since an increased basal T cell proliferation was reported to be associated to longer AT repeats (231). Thus, longer AT repeats in the 3'-UTR of the CD152 gene could impair the expression of the protein, and negatively affect the abolishment of an immune response. This would lead to a more active immune reaction.

We also wanted to investigate the other two well-known polymorphisms in the CD152 gene. Neither the C/T SNP in the promoter nor the A/G SNP in CDS1 were associated to MG in general. However, grouping the MG patients based on thymic histology, showed a higher frequency of the G allele or the G/G genotype in patients with thymoma compared to patients with normal thymus or thymic hyperplasia (Table 1, paper II). The G allele at position +49 in CDS1 of the CD152 gene is associated to a decreased inhibitory function of CD152 in Grave's disease, and suggested to be involved in the pathogenesis of the disease (232). In accordance with this, we detected a more active immune response in patients with the G/G genotype, manifested as elevated levels of $CD3^+CD28^+$ T cells and serum IL-1β (Fig 1a and 2a, paper II). Normally, almost all $CD4^+$ cells express CD28, but its expression varies on $CD8^+$ cells (233). It is therefore possible that the difference in expression lies in the $CD8^+$ compartment. Non-antigen specific $CD8^+$ regulatory cells show suppressive activity and originate from $CD8^+CD28^-$ cells (234, 235). If these $CD8^+$ regulatory cells are less frequent in persons with the G/G genotype, these persons might be more prone to develop autoimmune diseases. It would be interesting to repeat the experiment staining for more markers on T cells. It is also possible that the decreased levels of $CD3^+CD28^+$ cells are due to a down-regulation of the CD28 molecule, which can occur after repeated or continuous stimulation (reviewed in (236, 237)). In addition to the above described potential effects of the known CD152 polymorphisms, a T at the

polymorphic site at -318 in the promoter region led to increased promoter activity (238). Thus genetic differences in the CD152 gene can greatly influence the function of the protein and have devastating effects in a person who is prone to develop an autoimmune disease. This further suggests that the CD152 gene and its products have a great impact on the immune response.

The presence of the G allele, in addition to a longer AT repeat, could aggravate disease development in thymoma patients by influencing the expression or function of the CD152 gene and its products. An improper function of CD152 could explain the more severe disease with inflammatory infiltrates in the skeletal muscles often seen in thymoma patients (239).

4.4 ABNORMAL EXPRESSION OF CD152 (PAPER III)

MG is not associated with inflammation as some other autoimmune diseases like rheumatoid arthritis, multiple sclerosis, diabetes and SLE. However, a T cell response has to occur for the production of the pathogenic autoantibodies to take place. Since the T cell needs two signals to become activated, one via the TCR and one via costimulatory molecules, it is natural to investigate the costimulatory molecules and their expression in MG patients. We have shown that the total expression of CD152 in T cells (surface plus intracellular expression) was lower in MG patients compared to healthy controls, while no differences were seen at the mRNA level (Fig 1, paper III). In contrast, cells from patients with other diseases like Wegener's granulomatosis, SLE, multiple myeloma, Kawasaki disease, EBV infectious mononucleosis, malaria, B cell chronic lymphocytic leukemia, HIV and synovial fluid T cells from rheumatoid arthritis patients showed increased expression of CD152 (240-247). The discrepancy in CD152 expression in MG and other diseases might be due to differences in disease characteristics, for example genetic variants. In agreement with our study, CD152 expression was up-regulated in animal models of MG in association with different treatments (122, 123, 248). Thus, if the expression of CD152 could be up-regulated in MG patients, their condition might improve.

After stimulation of cells with ConA, cells from MG patients expressed lower levels of CD152 than cells from healthy controls (Fig 2 A, paper III). Other studies have shown an impaired up-regulation of CD152 in T cells after stimulation with PHA in patients with Wegener's granulomatosis and HIV, and after PMA plus ionomycin stimulation in SLE patients (240, 241, 246). On the other hand, cells from patients with B cell chronic lymphocytic leukemia demonstrated increased up-regulation of CD152 after anti-CD3 and IL-2 stimulation (243). An impaired up-regulation of CD152 on the T cells could explain an aberrant T cell function with a reduced possibility to turn off an ongoing immune response, and an immune system more easily activated. This may affect the disease outcome in autoimmune diseases like MG, SLE and Wegener's granulomatosis (240, 241, 246). In cancer patients on the other hand, the up-regulation of CD152 might lead to hyporesponsiveness or anergy as suggested in a publication (243). In addition, interference with the function of CD152 by anti-CD152 antibodies worsened murine EAMG (212), again pointing to the importance of this molecule in MG.

4.5 SOLUBLE COSTIMULATORY FACTORS IN MG PATIENTS (PAPER IV)

In recent years soluble forms of the costimulatory molecules CD28, CD80, CD86 and CD152 have been detected in humans (182-185). These factors are increased in some autoimmune diseases, malignancies and asthma (183, 184, 186-193). Their function is not completely elucidated, and the mechanism responsible for their release incompletely known. Due to the potential involvement of the costimulatory factors in the start or propagation of the disease, we wanted to determine if MG patients have altered levels of the soluble forms of these costimulatory factors. MG patients had increased serum levels of sCD152 compared to controls, and the increase was more pronounced in patients with thymoma (Fig 3 A and B, paper III). The levels of sCD152 were correlated to the levels of anti-AChR antibodies. However, in a following study in which we used other controls and not exactly the same patient group, there was no difference in the levels of sCD152 between healthy persons and MG patients, and no correlation to AChR antibody levels (data not shown, paper IV). This could be due to differences in the populations studied, or that the MG patients in the latter study were under better disease control. We could not detect any difference in the levels of the other soluble costimulatory factors sCD28, sCD80 or sCD86 between MG patients and controls. Some patients had lower levels of the soluble costimulatory molecules when they were in a "better disease stage", for example seen in the patient treated with anti-CD25 (Fig 6 A-D, paper VI). However, when we looked at 21 patients with MG sampled at different disease states, we could not demonstrate any difference (Table 3, paper VI). In fact, most patients seemed to have a rather constant expression of their soluble costimulatory factors despite changes in clinical stage.

The soluble costimulatory factors were correlated to one another (Table 4 and 5, paper IV). We could expect this since they probably are able to bind to each other in the circulation, and/or their release might be triggered by the same factors. To determine the usefulness of these soluble costimulatory factors as markers of disease activity, we measured other factors of immune activation as well. The soluble costimulatory factors were correlated to the cytokines IFN-γ, IL-6 and IL-10 in both MG patients and healthy controls, and correlated to sCD25 in healthy persons (Table 4 and 5, paper IV). sCD80 and sCD86 were correlated to sCD25 in the MG group as well, while this correlation was lost for sCD28 and sCD152 in the MG group (Table 4 and 5, paper IV). The lack of correlation of sCD28 and sCD152 to sCD25 in patients may reflect a difference in the release timing of the different proteins (see below).

sICAM-1 and sCD25 were increased in MG patients, findings reported also by others (Fig 1, paper IV) (249-251). The increase in sICAM-1 and sCD25 might be late events in the immune response, while the soluble costimulatory factors may be released early upon activation. This is supported by the fact that the mRNA splice variants of soluble CD28 and sCD152 are present in freshly prepared T cells, but disappear or decrease upon activation (252, 253). The presence of sCD80 and sCD86 is more complex since APCs preferentially express these molecules in membrane bound form, but T cells can also express them upon activation (160, 165, 166). The alternatively spliced sCD86 transcript was present in monocytes and peripheral blood dendritic cells, but not in T cells or NK cells (192). In the study by Hock et al. it was not elucidated if activated T

cells and NK cells express the mRNA for sCD86. Unstimulated B cells and monocytes, as well as activated T cells, express one form of sCD80 (lacking the transmembrane region), while unstimulated monocytes in addition to activated monocytes and activated T cells express a shorter form of sCD80 (lacking the IgC-like domain in addition to the transmembrane domain) (Fig 2, paper V) (illustrative picture, Fig 4). Thus, it is likely that monocytes and B cells secrete sCD80 upon activation, while T cells secrete the protein later after activation. This could explain the correlation of sCD80 and sCD86 to sCD25 in the MG patients; the T cells are constantly activated and hence secrete these proteins, while they only secrete sCD28 and sCD152 early in the immune response.

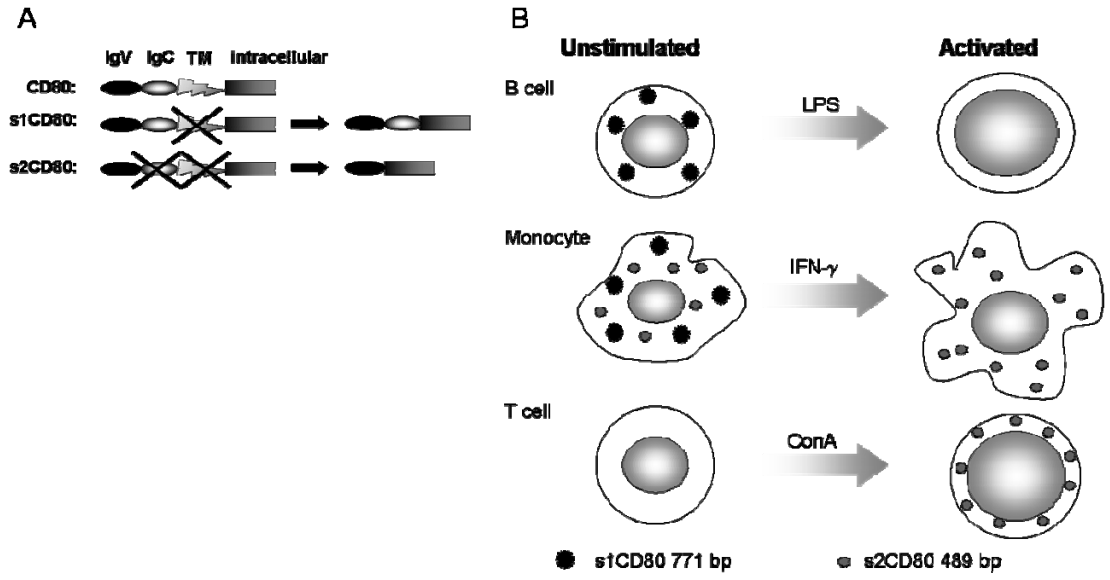

Fig 4. Soluble CD80 splice variants are expressed in various cells under different culture conditions. A) s1CD80 is a 771 bp long splice product lacking the transmembrane region of full length CD80, while s2CD80 is 480 bp long and lacks both the transmembrane and the IgC like domain. B) s1CD80 is expressed in unstimulated monocytes and B cells, while s2CD80 is expressed in unstimulated monocytes, as well as in stimulated monocytes and T cells.

4.6 SOLUBLE CD80 – A FUTURE DRUG CANDIDATE?

As we started to investigate the soluble costimulatory molecules in MG patients, there were no reports about the existence of human sCD80. We wanted to elucidate the function of this molecule since it has a complex binding feature – it can bind to both CD28 and CD152 mediating a signal for activation or inhibition, respectively. However, a soluble form of a membrane bound protein might have a different function since it does not have to cross-link its ligand and transduce a signal into the cell. It could rather function as a blocking agent and interfere with the binding of the membrane proteins. We observed that special immune cells expressed soluble CD80 splice variants under different culture conditions (Fig 2 B, paper V) (Fig 3). We produced one of these splice variants, s1CD80 lacking the transmembrane region (Fig 2 A, paper V, and illustrative picture Fig 4), in *E. coli* as a recombinant protein with a histidine tag and studied its function. The recombinant protein could bind to CD28 and

CD152 in ELISAs (Fig 4 A, paper V). It had a potent immunoregulatory function since it 1) preferentially interacted with activated T cells (Fig 4 B I, paper V, and Fig 5), 2) inhibited the mixed lymphocyte reaction as well as T cell proliferation induced by anti-CD3 (Fig 5 A and B, paper V), and 3) altered the cytokine secretion balance in the culture system (Fig 5 C-E, paper V). Additional studies support an immunoinhibitory role of soluble CD80 derivatives in human cells (200, 204), while one study showed the opposite (203). Discrepancies may be due to the different forms of the protein used, for example soluble derivatives consisting of the extracellular part only, or CD80Ig fusion proteins. A protein like soluble CD80, with a promising immunoinhibitory capacity, might be attractive as a potential drug candidate. However, its function *in vivo* has to be clarified. CTLA4-Ig has successfully been used to treat rheumatoid arthritis (254) and it is a potent inhibitor of immune activation *in vitro* as well as in animal studies (169, 195). Since this molecule binds to APCs it could prevent the activation of cells during an infection, not only hindering the autoimmune cells from becoming activated. An agent that directly targets the T cells, and preferentially the activated T cells, would be more beneficial since it still could allow for presentation of pathogenic agents entering the system.

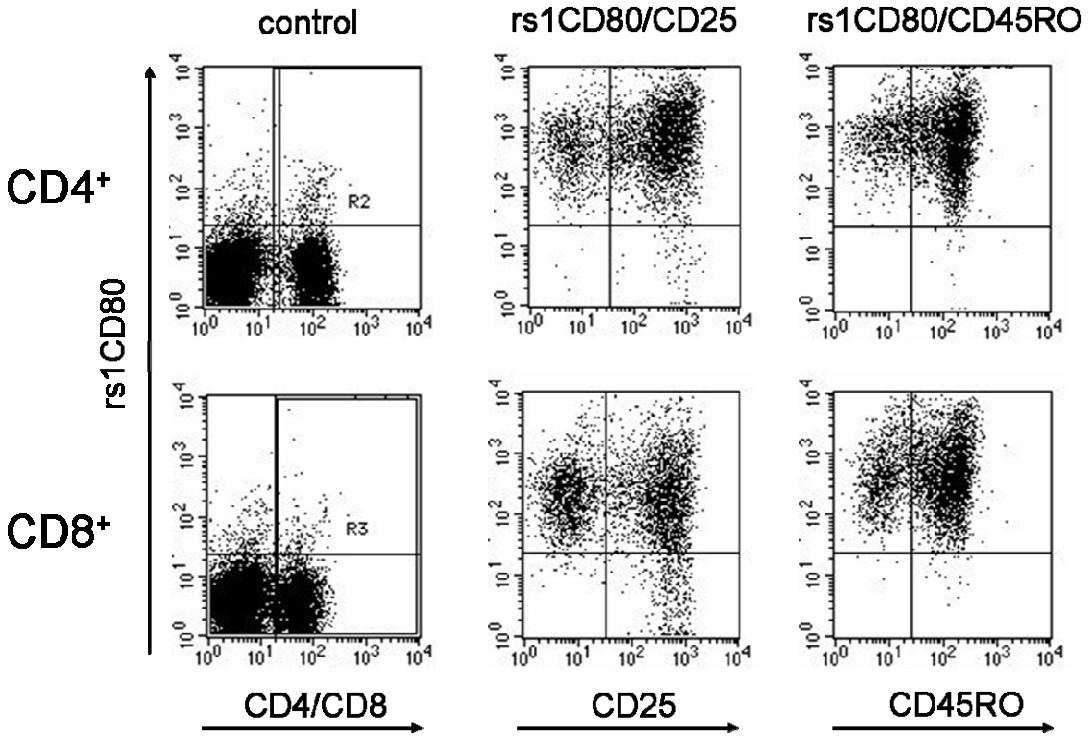

Fig 5. Rs1CD80 interacts preferentially with activated T cells. Representative dot plots showing the binding of Rs1CD80 to PHA activated PBMCs. Staining was performed using a biotinylated polyclonal antibody against CD80 and avidin conjugated to phycoerythrin. Control staining was done using the biotinylated polyclonal antibody and avidin-phycoerythrin. $CD25^+$ and $CD45RO^+$ cells represent activated T cells.

5 HYPOTHESES

5.1 THE START OF AN AUTOIMMUNE DISEASE

A complex disease like MG will not have a simple answer to the question "why does the body's immune system start an attack against a harmless self-antigen and how can we suppress the disease?". Thus, the scenario presented below is a simplified view of how the autoimmune reaction can start, with considerations to the findings in this study.

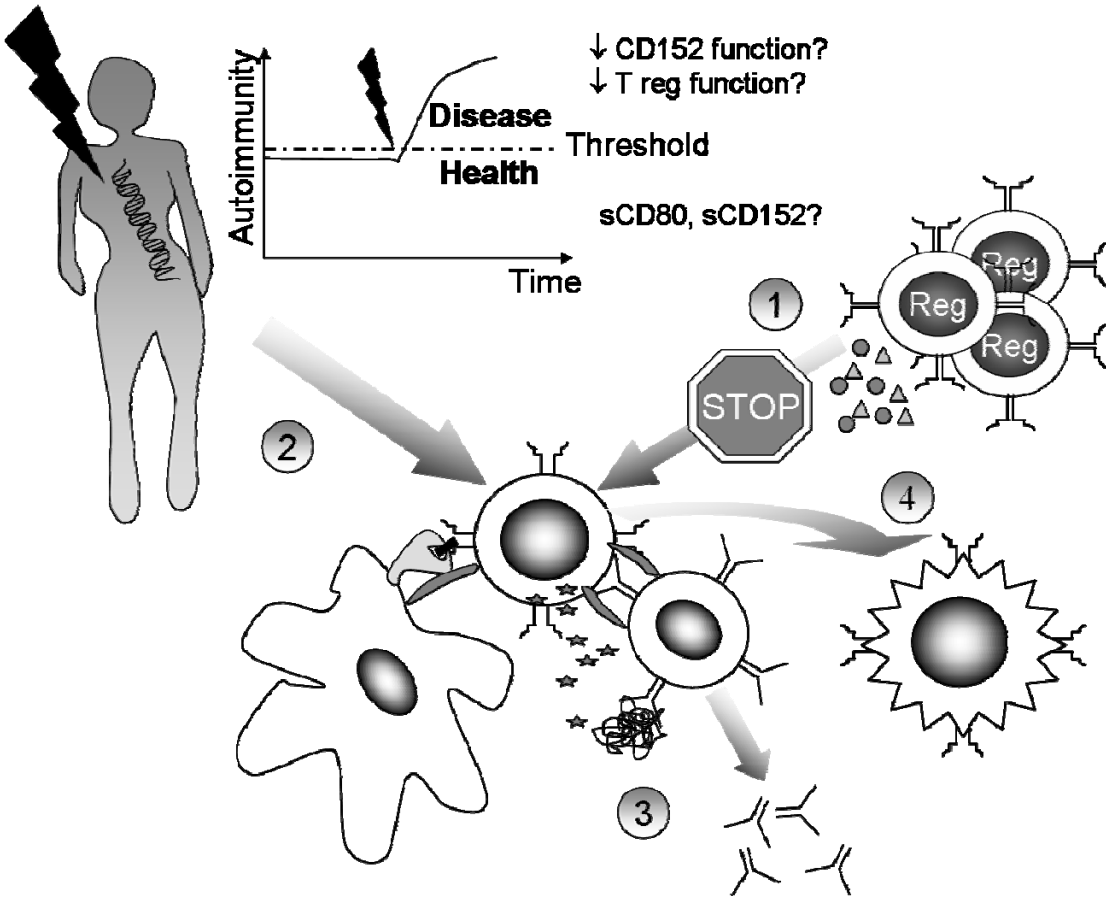

Fig 6. The start of an autoimmune disease like MG. (1) In a normal state, regulatory cells and inhibitory cytokines suppress the autoreactive cells. (2) However, an autoimmune disease may start if a genetically predisposed person is subjected to an environmental trigger, with activation of T cells pushing the immune system over the threshold for autoimmunity. (3) Activated autoreactive T cells will help B cells to produce antibodies against the autoantigen. (4) The aggressive T cells will make the disease progress. In MG, a decreased function of CD152 or regulatory T cells might predispose for the disease, while some soluble costimulatory factors if present could possibly help to prevent disease.

Something occurs in a person with a genetic predisposition for the disease that makes the person start to develop MG (Fig 6). A decreased function or expression of CD152 might favor disease development, although it alone does not account for the start of the disease. Other factors, like a reduced function of regulatory cells could have an effect. The activated T cells will help B cells to become plasma cells and secrete antibodies.

Aggressive T cells will probably also drive the disease further. Since deletion or abolishment of T cell function has a beneficial effect on the disease, and a loss of AChR specific cytokine responses is associated with lack of disease, the T cells could be used as drug targets. However, even though deleting all T cells or interfering with all cells expressing CD25 has a beneficial effect on the disease, these therapies are too unspecific. Interfering with the costimulatory molecules, for example by supplying soluble CD80 to a patient, could be a way to target only the activated T cells, and increase the threshold of activation.

5.2 THE FUNCTION OF SOLUBLE CD80 AND SOLUBLE CD152

As the function of the soluble costimulatory factors is not fully clear, a very speculative theory of their action is proposed below.

The soluble forms of the costimulatory molecules could play different roles: 1) the soluble factors could bind to their soluble counter ligands and prevent them from degradation before they bind to their membrane bound receptors; 2) they could bind to their membrane bound counter ligands and induce a signal or 3) hinder the binding of the membrane bound molecules. The complexity of this network is shown by the diversity of the reports using the soluble proteins in experimental studies. It is possible that some of the soluble costimulatory factors are stimulatory while others are inhibitory, although they might even have the same net outcome. Since reports have demonstrated conflicting results on the impact of these molecules on T cell activation, I will simplify the scenario and suggest a hypothesis where sCD80 and sCD152 have a negative impact on the immune system (I will not consider sCD28 and sCD86 since the chimeric proteins of these molecules activate the immune response in animal studies).

If the soluble costimulatory molecules are just blocking their respective receptors, a strong enough signal via the TCR would still lead to T cell activation (eg if an infection occurs). On the other hand, when no infection occurs, the molecules may just keep the threshold for activation at a higher level, favoring tolerance. A difference in affinity for the membrane bound and soluble forms could also play a role. Suppose that sCD80 is bound to a T cell. An APC expressing membrane bound CD80 starts to interact with the T cell. If soluble CD80 has a lower affinity to its ligand compared to membrane bound CD80, the latter will push the soluble form away from the cell.

A preferential finding of the mRNA for the soluble form of CD152 in resting cells, indicates a release of the soluble protein upon activation. This would lead to an overall suppression of nearby cells in regard to the start of an immune response (Fig 7). Later on, when the B cells are activated, they release s1CD80, which has the potential to suppress activation of neighboring T cells (our *in vitro* experiments). Monocytes may also secrete s1CD80 upon activation and increase the threshold of activation for cells in the vicinity. Naïve and activated monocytes, in addition to activated T cells, express s2CD80. However, at this point we do not know what function this protein has – or if it has any function. To summarize, I believe that the soluble costimulatory factors sCD80 and sCD152 are immunosuppressive, and that their up-regulation in different disease states is a sign that the immune system is trying to down-regulate an active response.

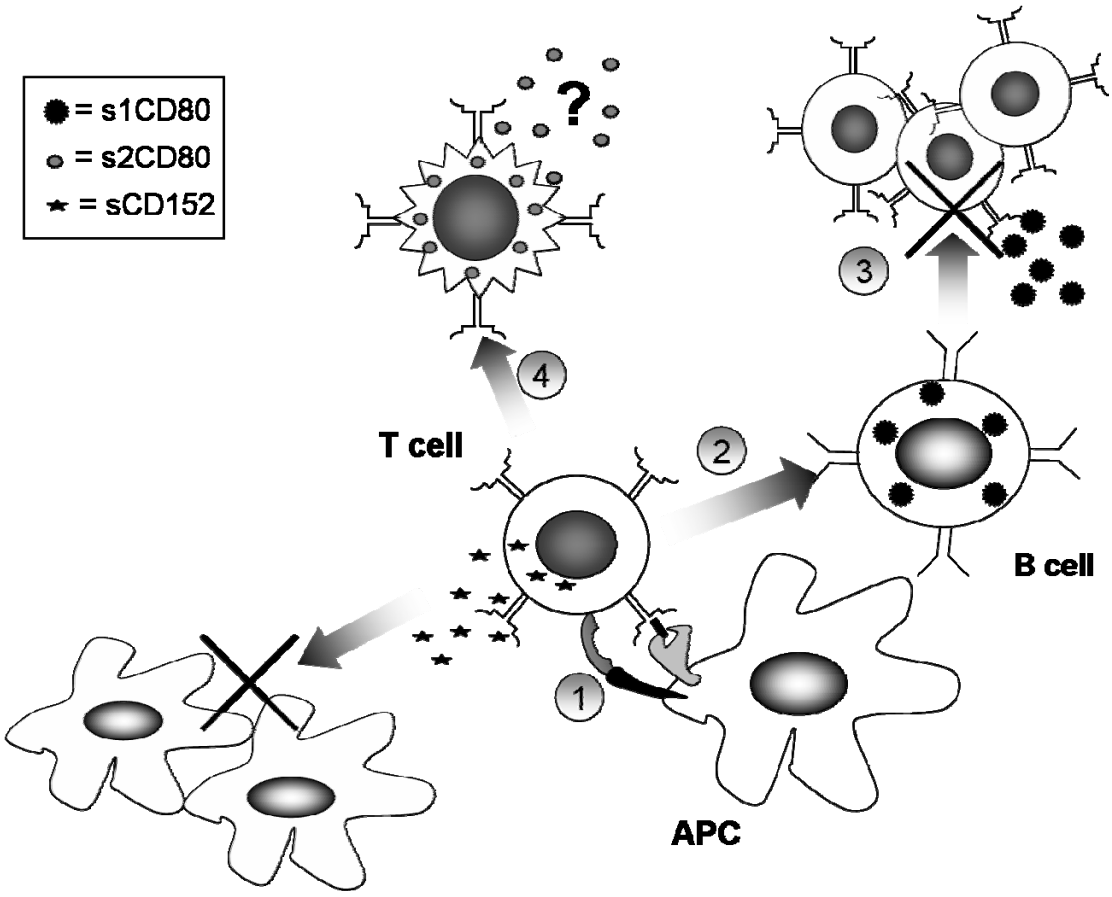

Fig 7. A hypothetical view of the function of sCD80 and sCD152. A splice form of sCD152 is expressed in unstimulated T cells at the mRNA level. 1) Upon activation, here illustrated by interaction with an APC, the cell releases sCD152. The protein might have a suppressive effect on neighboring APCs. 2) The activated T cell will help to activate the B cell, which will release its content of s1CD80. 3) s1CD80 has an inhibitory effect on T cell activation, hindering T cells in the vicinity to become activated. 4) Activated T cells express s2CD80, although its function has not been elucidated yet. Monocytes are not shown in the picture. Unstimulated monocytes express both s1CD80 and s2CD80, while they upon activation only express the short form – s2CD80. s1CD80 secreted by monocytes may also increase the threshold of activation for nearby cells, assuring that only those T cells with a true specificity for the antigen will be activated.

6 CONCLUSIONS

- The T cells are important in the initiation and the maintenance of the immune response against the AChR in MG. The healthy twins in two twin pairs discordant for MG did not have T cell responses against the AChR, but they had pathogenic antibodies normally not seen in healthy persons. A patient treated with antibodies targeting the CD25 protein, preferentially expressed on T cells, improved in the disease. The activated cells and humoral factors associated to a potential immune activity decreased, while naïve cells increased, indicating a normalization of the immune system. These results together suggest that T cells have a major role in MG.

- Genetic aberrancies in genes important for proper T cell function can influence disease susceptibility. Genetic variants of the CD152 gene could influence the down-regulation of an immune response. MG patients with thymoma demonstrated genetic aberrancies in the CD152 gene, which might contribute to the more aggressive disease seen in this group of patients. Moreover, MG patients displayed reduced CD152 expression on T cells, suggesting a defective inhibition of T cell activation.

- The regulation of costimulatory molecules and their derivatives might help to combat autoimmune diseases like MG. However, the levels of the soluble costimulatory factors were not altered in MG patients compared to healthy controls, and the levels seemed to be stable in individual patients independent of clinical presentation.

- The recombinant form of naturally occurring soluble CD80 inhibited immune activation and preferentially interacted with activated T cells, suggesting that it might be used as a drug to suppress ongoing T cell activation.

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8 REFERENCES

1. Simpson, J. A. 1960. Myasthenia gravis, a new hypothesis. *Scott Med J 5:419*.
2. Nastuk, W. L., A. J. Strauss, and K. E. Osserman. 1959. Search for a neuromuscular blocking agent in the blood of patients with myasthenia gravis. *Am J Med 26:394*.
3. Fambrough, D. M., D. B. Drachman, and S. Satyamurti. 1973. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science 182:293*.
4. Engel, A. G., J. M. Lindstrom, E. H. Lambert, and V. A. Lennon. 1977. Ultrastructural localization of the acetylcholine receptor in myasthenia gravis and in its experimental autoimmune model. *Neurology 27:307*.
5. Tzartos, S. J., T. Barkas, M. T. Cung, A. Mamalaki, M. Marraud, P. Orlewski, D. Papanastasiou, C. Sakarellos, M. Sakarellos-Daitsiotis, P. Tsantili, and V. Tsikaris. 1998. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunol Rev 163:89*.
6. Kalb, B., G. Matell, R. Pirskanen, and M. Lambe. 2002. Epidemiology of myasthenia gravis: a population-based study in Stockholm, Sweden. *Neuroepidemiology 21:221*.
7. Oosterhuis, H. J. 1981. Myasthenia gravis. A survey. *Clin Neurol Neurosurg 83:105*.
8. Aarli, J. A. 1999. Late-onset myasthenia gravis: a changing scene. *Arch Neurol 56:25*.
9. Oosterhuis, H. J. 1988. Long-term effects of treatment in 374 patients with myasthenia gravis. *Monogr Allergy 25:75*.
10. Elliott, M. J., R. N. Maini, M. Feldmann, J. R. Kalden, C. Antoni, J. S. Smolen, B. Leeb, F. C. Breedveld, J. D. Macfarlane, H. Bijl, and et al. 1994. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet 344:1105*.
11. Elliott, M. J., R. N. Maini, M. Feldmann, A. Long-Fox, P. Charles, P. Katsikis, F. M. Brennan, J. Walker, H. Bijl, J. Ghrayeb, and et al. 1993. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum 36:1681*.
12. Rowin, J., M. N. Meriggioli, E. Tuzun, S. Leurgans, and P. Christadoss. 2004. Etanercept treatment in corticosteroid-dependent myasthenia gravis. *Neurology 63:2390*.
13. Zaja, F., D. Russo, G. Fuga, G. Perella, and M. Baccarani. 2000. Rituximab for myasthenia gravis developing after bone marrow transplant. *Neurology 55:1062*.
14. Wylam, M. E., P. M. Anderson, N. L. Kuntz, and V. Rodriguez. 2003. Successful treatment of refractory myasthenia gravis using rituximab: a pediatric case report. *J Pediatr 143:674*.
15. Takagi, K., A. Yoshida, H. Iwasaki, H. Inoue, and T. Ueda. 2005. Anti-CD20 antibody (Rituximab) therapy in a myasthenia gravis patient with follicular lymphoma. *Ann Hematol*.
16. Gajra, A., N. Vajpayee, and S. J. Grethlein. 2004. Response of myasthenia gravis to rituximab in a patient with non-Hodgkin lymphoma. *Am J Hematol 77:196*.
17. Kovarik, J. M., E. Rawlings, P. Sweny, O. Fernando, R. Moore, P. J. Griffin, P. Fauchald, D. Albrechtsen, G. Sodal, K. Nordal, and P. L. Amlot. 1996. Prolonged immunosuppressive effect and minimal immunogenicity from chimeric (CD25) monoclonal antibody SDZ CHI 621 in renal transplantation. *Transplant Proc 28:913*.
18. Amlot, P. L., E. Rawlings, O. N. Fernando, P. J. Griffin, G. Heinrich, M. H. Schreier, J. P. Castaigne, R. Moore, and P. Sweny. 1995. Prolonged action of a

chimeric interleukin-2 receptor (CD25) monoclonal antibody used in cadaveric renal transplantation. *Transplantation 60:748*.
19. Mrowietz, U., K. Zhu, and E. Christophers. 2000. Treatment of severe psoriasis with anti-CD25 monoclonal antibodies. *Arch Dermatol 136:675*.
20. Bell, H. K., and R. A. Parslew. 2002. Use of basiliximab as a cyclosporin-sparing agent in palmoplantar pustular psoriasis with myalgia as an adverse effect. *Br J Dermatol 147:606*.
21. Owen, C. M., and P. V. Harrison. 2000. Successful treatment of severe psoriasis with basiliximab, an interleukin-2 receptor monoclonal antibody. *Clin Exp Dermatol 25:195*.
22. Salim, A., R. M. Emerson, and K. L. Dalziel. 2000. Successful treatment of severe generalized pustular psoriasis with basiliximab (interleukin-2 receptor blocker). *Br J Dermatol 143:1121*.
23. Kagi, M. K., and G. Heyer. 2001. Efficacy of basiliximab, a chimeric anti-interleukin-2 receptor monoclonal antibody, in a patient with severe chronic atopic dermatitis. *Br J Dermatol 145:350*.
24. Rebora, A., A. Parodi, and G. Murialdo. 2002. Basiliximab is effective for erosive lichen planus. *Arch Dermatol 138:1100*.
25. Haufs, M. G., and E. Haneke. 2001. Epidermolysis bullosa acquisita treated with basiliximab, an interleukin-2 receptor antibody. *Acta Derm Venereol 81:72*.
26. Berman, J. A., K. Patel, and J. Caro. 2002. Enhanced response to basiliximab in a patient with aplastic anemia after treatment with standard immunosuppression. *Am J Hematol 71:64*.
27. Nussenblatt, R. B., E. Fortin, R. Schiffman, L. Rizzo, J. Smith, P. Van Veldhuisen, P. Sran, A. Yaffe, C. K. Goldman, T. A. Waldmann, and S. M. Whitcup. 1999. Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. *Proc Natl Acad Sci U S A 96:7462*.
28. Nussenblatt, R. B., D. J. Thompson, Z. Li, C. C. Chan, J. S. Peterson, R. R. Robinson, R. S. Shames, S. Nagarajan, M. T. Tang, M. Mailman, G. Velez, C. Roy, G. A. Levy-Clarke, E. B. Suhler, A. Djalilian, H. N. Sen, S. Al-Khatib, R. Ursea, S. Srivastava, A. Bamji, S. Mellow, P. Sran, T. A. Waldmann, and R. R. Buggage. 2003. Humanized anti-interleukin-2 (IL-2) receptor alpha therapy: long-term results in uveitis patients and preliminary safety and activity data for establishing parameters for subcutaneous administration. *J Autoimmun 21:283*.
29. Creed, T. J., M. R. Norman, C. S. Probert, R. F. Harvey, I. S. Shaw, J. Smithson, J. Anderson, M. Moorghen, J. Gupta, N. A. Shepherd, C. M. Dayan, and S. D. Hearing. 2003. Basiliximab (anti-CD25) in combination with steroids may be an effective new treatment for steroid-resistant ulcerative colitis. *Aliment Pharmacol Ther 18:65*.
30. Bielekova, B., N. Richert, T. Howard, G. Blevins, S. Markovic-Plese, J. McCartin, J. A. Frank, J. Wurfel, J. Ohayon, T. A. Waldmann, H. F. McFarland, and R. Martin. 2004. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. *Proc Natl Acad Sci U S A 101:8705*.
31. Rose, J. W., H. E. Watt, A. T. White, and N. G. Carlson. 2004. Treatment of multiple sclerosis with an anti-interleukin-2 receptor monoclonal antibody. *Ann Neurol 56:864*.
32. Brok, H. P., J. M. Tekoppele, J. Hakimi, J. A. Kerwin, E. M. Nijenhuis, C. W. De Groot, R. E. Bontrop, and B. A. t Hart. 2001. Prophylactic and therapeutic effects of a humanized monoclonal antibody against the IL-2 receptor (DACLIZUMAB) on collagen-induced arthritis (CIA) in rhesus monkeys. *Clin Exp Immunol 124:134*.
33. Allen, N., P. Kissel, D. Pietrasiuk, and M. J. Perlow. 1984. Myasthenia gravis in monozygotic twins. Clinical follow-up nine years after thymectomy. *Arch Neurol 41:994*.
34. Behan, P. O. 1980. Immune disease and HLA associations with myasthenia gravis. *J Neurol Neurosurg Psychiatry 43:611*.

35. Murphy, J., and S. F. Murphy. 1986. Myasthenia gravis in identical twins. *Neurology 36:78*.
36. Namba, T., N. G. Brunner, S. B. Brown, M. Muguruma, and D. Grob. 1971. Familial myasthenia gravis. Report of 27 patients in 12 families and review of 164 patients in 73 families. *Arch Neurol 25:49*.
37. Leslie, R. D., and M. Hawa. 1994. Twin studies in auto-immune disease. *Acta Genet Med Gemellol 43:71*.
38. Garchon, H. J. 2003. Genetics of autoimmune myasthenia gravis, a model for antibody-mediated autoimmunity in man. *J Autoimmun 21:105*.
39. Huang, D., R. Pirskanen, P. Hjelmstrom, and A. K. Lefvert. 1998. Polymorphisms in IL-1beta and IL-1 receptor antagonist genes are associated with myasthenia gravis. *J Neuroimmunol 81:76*.
40. Huang, D., L. Liu, K. Noren, S. Q. Xia, J. Trifunovic, R. Pirskanen, and A. K. Lefvert. 1998. Genetic association of Ctla-4 to myasthenia gravis with thymoma. *J Neuroimmunol 88:192*.
41. Huang, D. R., Y. H. Zhou, S. Q. Xia, L. Liu, R. Pirskanen, and A. K. Lefvert. 1999. Markers in the promoter region of interleukin-10 (IL-10) gene in myasthenia gravis: implications of diverse effects of IL-10 in the pathogenesis of the disease. *J Neuroimmunol 94:82*.
42. Huang, D. R., R. Pirskanen, G. Matell, and A. K. Lefvert. 1999. Tumour necrosis factor-alpha polymorphism and secretion in myasthenia gravis. *J Neuroimmunol 94:165*.
43. Hjelmstrom, P., C. S. Peacock, R. Giscombe, R. Pirskanen, A. K. Lefvert, J. M. Blackwell, and C. B. Sanjeevi. 1998. Polymorphism in tumor necrosis factor genes associated with myasthenia gravis. *J Neuroimmunol 88:137*.
44. Heckmann, J. M., K. E. Morrison, B. Emeryk-Szajewska, H. Strugalska, J. Bergoffen, N. Willcox, and J. Newsom-Davis. 1996. Human muscle acetylcholine receptor alpha-subunit gene (CHRNA1) association with autoimmune myasthenia gravis in black, mixed-ancestry and Caucasian subjects. *J Autoimmun 9:175*.
45. Giraud, M., G. Beaurain, B. Eymard, C. Tranchant, P. Gajdos, and H. J. Garchon. 2004. Genetic control of autoantibody expression in autoimmune myasthenia gravis: role of the self-antigen and of HLA-linked loci. *Genes Immun 5:398*.
46. Garchon, H. J., F. Djabiri, J. P. Viard, P. Gajdos, and J. F. Bach. 1994. Involvement of human muscle acetylcholine receptor alpha-subunit gene (CHRNA) in susceptibility to myasthenia gravis. *Proc Natl Acad Sci U S A 91:4668*.
47. Xu, B. Y., D. Huang, R. Pirskanen, and A. K. Lefvert. 2000. beta2-adrenergic receptor gene polymorphisms in myasthenia gravis (MG). *Clin Exp Immunol 119:156*.
48. Nakao, Y., H. Matsumoto, T. Miyazaki, H. Nishitani, K. Ota, T. Fujita, and K. Tsuji. 1980. Gm allotypes in myasthenia gravis. *Lancet 1:677*.
49. Smith, C. I., R. Grubb, L. Hammarstrom, and R. Pirskanen. 1984. Gm allotypes in Finnish myasthenia gravis patients. *Neurology 34:1604*.
50. Chiu, H. C., G. G. de Lange, N. Willcox, A. Vincent, J. Newsom-Davis, K. H. Hsieh, and T. P. Hung. 1988. Immunoglobulin allotypes in caucasian and Chinese myasthenia gravis: differences from Japanese patients. *J Neurol Neurosurg Psychiatry 51:214*.
51. Gigliotti, D., A. K. Lefvert, M. Jeddi-Tehrani, S. Esin, V. Hodara, R. Pirskanen, H. Wigzell, and R. Andersson. 1996. Overexpression of select T cell receptor V beta gene families within CD4+ and CD8+ T cell subsets of myasthenia gravis patients: a role for superantigen(s)? *Mol Med 2:452*.
52. Mantegazza, R., J. R. Oksenberg, F. Baggi, C. Antozzi, M. T. Illeni, G. Pellegris, F. Cornelio, and L. Steinman. 1990. Increased incidence of certain TCR and HLA genes associated with myasthenia gravis in Italians. *J Autoimmun 3:431*.
53. Oksenberg, J. R., M. Sherritt, A. B. Begovich, H. A. Erlich, C. C. Bernard, L. L. Cavalli-Sforza, and L. Steinman. 1989. T-cell receptor V alpha and C alpha

alleles associated with multiple and myasthenia gravis. *Proc Natl Acad Sci U S A 86:988*.
54. Oksenberg, J. R., C. N. Gaiser, L. L. Cavalli-Sforza, and L. Steinman. 1988. Polymorphic markers of human T-cell receptor alpha and beta genes. Family studies and comparison of frequencies in healthy individuals and patients with multiple sclerosis and myasthenia gravis. *Hum Immunol 22:111*.
55. Raknes, G., G. O. Skeie, N. E. Gilhus, S. Aadland, and C. Vedeler. 1998. FcgammaRIIA and FcgammaRIIIB polymorphisms in myasthenia gravis. *J Neuroimmunol 81:173*.
56. van der Pol, W. L., M. D. Jansen, J. B. Kuks, M. de Baets, F. G. Leppers-van de Straat, J. H. Wokke, J. G. van de Winkel, and L. H. van den Berg. 2003. Association of the Fc gamma receptor IIA-R/R131 genotype with myasthenia gravis in Dutch patients. *J Neuroimmunol 144:143*.
57. Kristiansen, O. P., Z. M. Larsen, and F. Pociot. 2000. CTLA-4 in autoimmune diseases--a general susceptibility gene to autoimmunity? *Genes Immun 1:170*.
58. Dariavach, P., M. G. Mattei, P. Golstein, and M. P. Lefranc. 1988. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. *Eur J Immunol 18:1901*.
59. Deichmann, K., A. Heinzmann, E. Bruggenolte, J. Forster, and J. Kuehr. 1996. An Mse I RFLP in the human CTLA4 promotor. *Biochem Biophys Res Commun 225:817*.
60. Nistico, L., R. Buzzetti, L. E. Pritchard, B. Van der Auwera, C. Giovannini, E. Bosi, M. T. Larrad, M. S. Rios, C. C. Chow, C. S. Cockram, K. Jacobs, C. Mijovic, S. C. Bain, A. H. Barnett, C. L. Vandewalle, F. Schuit, F. K. Gorus, R. Tosi, P. Pozzilli, and J. A. Todd. 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum Mol Genet 5:1075*.
61. Polymeropoulos, M. H., H. Xiao, D. S. Rath, and C. R. Merril. 1991. Dinucleotide repeat polymorphism at the human CTLA4 gene. *Nucleic Acids Res 19:4018.*
62. Castleman, B. 1966. The pathology of the thymus gland in myasthenia gravis. *Ann N Y Acad Sci 135:496*.
63. Levine, G. D., and J. Rosai. 1978. Thymic hyperplasia and neoplasia: a review of current concepts. *Hum Pathol 9:495*.
64. Oosterhuis, H. J. 1989. The natural course of myasthenia gravis: a long term follow up study. *J Neurol Neurosurg Psychiatry 52:1121*.
65. Schonbeck, S., F. Padberg, R. Hohlfeld, and H. Wekerle. 1992. Transplantation of thymic autoimmune microenvironment to severe combined immunodeficiency mice. A new model of myasthenia gravis. *J Clin Invest 90:245*.
66. Simpson, J. A. 1958. An evaluation of thymectomy in myasthenia gravis. *Brain 81:112*.
67. Jaretzki, A., K. M. Steinglass, and J. R. Sonett. 2004. Thymectomy in the management of myasthenia gravis. *Semin Neurol 24:49*.
68. Kirchner, T., S. Tzartos, F. Hoppe, B. Schalke, H. Wekerle, and H. K. Muller-Hermelink. 1988. Pathogenesis of myasthenia gravis. Acetylcholine receptor-related antigenic determinants in tumor-free thymuses and thymic epithelial tumors. *Am J Pathol 130:268*.
69. Schluep, M., N. Willcox, A. Vincent, G. K. Dhoot, and J. Newsom-Davis. 1987. Acetylcholine receptors in human thymic myoid cells in situ: an immunohistological study. *Ann Neurol 22:212*.
70. Melms, A., B. C. Schalke, T. Kirchner, H. K. Muller-Hermelink, E. Albert, and H. Wekerle. 1988. Thymus in myasthenia gravis. Isolation of T-lymphocyte lines specific for the nicotinic acetylcholine receptor from thymuses of myasthenic patients. *J Clin Invest 81:902*.
71. Vincent, A., G. K. Scadding, H. C. Thomas, and J. Newsom-Davis. 1978. In-vitro synthesis of anti-acetylcholine-receptor antibody by thymic lymphocytes in myasthenia gravis. *Lancet 1:305*.

72. Sommer, N., N. Willcox, G. C. Harcourt, and J. Newsom-Davis. 1990. Myasthenic thymus and thymoma are selectively enriched in acetylcholine receptor-reactive T cells. *Ann Neurol 28:312*.
73. Kao, I., and D. B. Drachman. 1977. Thymic muscle cells bear acetylcholine receptors: possible relation to myasthenia gravis. *Science 195:74*.
74. Gilhus, N. E., J. A. Aarli, B. Christensson, and R. Matre. 1984. Rabbit antiserum to a citric acid extract of human skeletal muscle staining thymomas from myasthenia gravis patients. *J Neuroimmunol 7:55*.
75. Marx, A., H. K. Muller-Hermelink, and P. Strobel. 2003. The role of thymomas in the development of myasthenia gravis. *Ann N Y Acad Sci 998:223*.
76. Lindstrom, J. M., M. E. Seybold, V. A. Lennon, S. Whittingham, and D. D. Duane. 1976. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology 26:1054*.
77. Lefvert, A. K., K. Bergstrom, G. Matell, P. O. Osterman, and R. Pirskanen. 1978. Determination of acetylcholine receptor antibody in myasthenia gravis: clinical usefulness and pathogenetic implications. *J Neurol Neurosurg Psychiatry 41:394*.
78. Toyka, K. V., D. B. Brachman, A. Pestronk, and I. Kao. 1975. Myasthenia gravis: passive transfer from man to mouse. *Science 190:397*.
79. Toyka, K. V., D. B. Drachman, D. E. Griffin, A. Pestronk, J. A. Winkelstein, K. H. Fishbeck, and I. Kao. 1977. Myasthenia gravis. Study of humoral immune mechanisms by passive transfer to mice. *N Engl J Med 296:125*.
80. Patrick, J., and J. Lindstrom. 1973. Autoimmune response to acetylcholine receptor. *Science 180:871*.
81. Lennon, V. A., J. M. Lindstrom, and M. E. Seybold. 1975. Experimental autoimmune myasthenia: A model of myasthenia gravis in rats and guinea pigs. *J Exp Med 141:1365*.
82. Burges, J., D. W. Wray, S. Pizzighella, Z. Hall, and A. Vincent. 1990. A myasthenia gravis plasma immunoglobulin reduces miniature endplate potentials at human endplates in vitro. *Muscle Nerve 13:407*.
83. Tzartos, S. J., and A. Starzinski-Powitz. 1986. Decrease in acetylcholine-receptor content of human myotube cultures mediated by monoclonal antibodies to alpha, beta and gamma subunits. *FEBS Lett 196:91*.
84. Heinemann, S., S. Bevan, R. Kullberg, J. Lindstrom, and J. Rice. 1977. Modulation of acetylcholine receptor by antibody against the receptor. *Proc Natl Acad Sci U S A 74:3090*.
85. Engel, A. G., E. H. Lambert, and F. M. Howard. 1977. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. *Mayo Clin Proc 52:267*.
86. Burges, J., A. Vincent, P. C. Molenaar, J. Newsom-Davis, C. Peers, and D. Wray. 1994. Passive transfer of seronegative myasthenia gravis to mice. *Muscle Nerve 17:1393*.
87. Mossman, S., A. Vincent, and J. Newsom-Davis. 1986. Myasthenia gravis without acetylcholine-receptor antibody: a distinct disease entity. *Lancet 1:116*.
88. Vincent, A., J. McConville, M. E. Farrugia, and J. Newsom-Davis. 2004. Seronegative myasthenia gravis. *Semin Neurol 24:125*.
89. Vincent, A., J. Bowen, J. Newsom-Davis, and J. McConville. 2003. Seronegative generalised myasthenia gravis: clinical features, antibodies, and their targets. *Lancet Neurol 2:99*.
90. Aarli, J. A., K. Stefansson, L. S. Marton, and R. L. Wollmann. 1990. Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. *Clin Exp Immunol 82:284*.
91. Hoch, W., J. McConville, S. Helms, J. Newsom-Davis, A. Melms, and A. Vincent. 2001. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med 7:365*.
92. Mygland, A., O. B. Tysnes, R. Matre, P. Volpe, J. A. Aarli, and N. E. Gilhus. 1992. Ryanodine receptor autoantibodies in myasthenia gravis patients with a thymoma. *Ann Neurol 32:589*.

93. Ohta, M., K. Ohta, N. Itoh, M. Kurobe, K. Hayashi, and H. Nishitani. 1990. Anti-skeletal muscle antibodies in the sera from myasthenic patients with thymoma: identification of anti-myosin, actomyosin, actin, and alpha-actinin antibodies by a solid-phase radioimmunoassay and a western blotting analysis. *Clin Chim Acta 187:255*.
94. Williams, C. L., and V. A. Lennon. 1986. Thymic B lymphocyte clones from patients with myasthenia gravis secrete monoclonal striational autoantibodies reacting with myosin, alpha actinin, or actin. *J Exp Med 164:1043*.
95. Xu, B. Y., R. Pirskanen, and A. K. Lefvert. 1998. Antibodies against beta1 and beta2 adrenergic receptors in myasthenia gravis. *J Neuroimmunol 91:82*.
96. Eng, H., Y. Magnusson, G. Matell, A. K. Lefvert, R. Saponja, and J. Hoebeke. 1992. Beta 2-adrenergic receptor antibodies in myasthenia gravis. *J Autoimmun 5:213*.
97. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann Immunol (Paris) 125C:373*.
98. Wassermann, N. H., A. S. Penn, P. I. Freimuth, N. Treptow, S. Wentzel, W. L. Cleveland, and B. F. Erlanger. 1982. Anti-Idiotypic Route to Anti-Acetylcholine Receptor Antibodies and Experimental Myasthenia Gravis. *PNAS 79:4810*.
99. Cleveland, W. L., N. H. Wassermann, R. Sarangarajan, A. S. Penn, and B. F. Erlanger. 1983. Monoclonal antibodies to the acetylcholine receptor by a normally functioning auto-anti-idiotypic mechanism. *Nature 305:56*.
100. Lefvert, A. K., R. W. James, C. Alliod, and B. W. Fulpius. 1982. A monoclonal anti-idiotypic antibody against anti-receptor antibodies from myasthenic sera. *Eur J Immunol 12:790*.
101. Dwyer, D. S., R. J. Bradley, C. K. Urquhart, and J. F. Kearney. 1983. Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. *Nature 301:611*.
102. Lefvert, A. K. 1981. Anti-idiotypic antibodies against the receptor antibodies in myasthenia gravis. *Scand J Immunol 13:493*.
103. Yi, Q., and A. K. Lefvert. 1992. Idiotypic and antiidiotypic T and B lymphocytes in myasthenia gravis. *J Immunol 149:3423*.
104. Lefvert, A. K., and B. W. Fulpius. 1984. Receptor-like activity of a monoclonal anti-idiotypic antibody against an anti-acetylcholine receptor antibody. *Scand J Immunol 19:485*.
105. Lefvert, A. K. 1988. The start of an autoimmune disease: idiotypic network during early progression of myasthenia gravis. *Ann Inst Pasteur Immunol 139:633*.
106. Lefvert, A. K., R. Pirskanen, and E. Svanborg. 1985. Anti-idiotypic antibodies, acetylcholine receptor antibodies and disturbed neuromuscular function in healthy relatives to patients with myasthenia gravis. *J Neuroimmunol 9:41*.
107. Lefvert, A. K., and K. Bergstrom. 1978. Acetylcholine receptor antibody in myasthenia gravis: purification and characterization. *Scand J Immunol 8:525*.
108. Li, H., F. D. Shi, B. He, M. Bakheit, B. Wahren, A. Berglof, K. Sandstedt, and H. Link. 1998. Experimental autoimmune myasthenia gravis induction in B cell-deficient mice. *Int Immunol 10:1359*.
109. Dedhia, V., E. Goluszko, B. Wu, C. Deng, and P. Christadoss. 1998. The effect of B cell deficiency on the immune response to acetylcholine receptor and the development of experimental autoimmune myasthenia gravis. *Clin Immunol Immunopathol 87:266*.
110. De Baets, M. H., B. Einarson, J. M. Lindstrom, and W. O. Weigle. 1982. Lymphocyte activation in experimental autoimmune myasthenia gravis. *J Immunol 128:2228*.
111. Lennon, V. A., J. M. Lindstrom, and M. E. Seybold. 1976. Experimental autoimmune myasthenia gravis: cellular and humoral immune responses. *Ann N Y Acad Sci 274:283*.
112. Hohlfeld, R., K. V. Toyka, S. J. Tzartos, W. Carson, and B. M. Conti-Tronconi. 1987. Human T-Helper Lymphocytes in Myasthenia Gravis Recognize the Nicotinic Receptor alpha Subunit. *PNAS 84:5379*.

113. Hohlfeld, R., K. V. Toyka, K. Heininger, H. Grosse-Wilde, and I. Kalies. 1984. Autoimmune human T lymphocytes specific for acetylcholine receptor. *Nature 310:244*.
114. Abramsky, O., A. Aharonov, C. Webb, and S. Fuchs. 1975. Cellular immune response to acetylcholine receptor-rich fraction, in patients with myasthenia gravis. *Clin Exp Immunol 19:11*.
115. Richman, D. P., J. Patrick, and B. G. Arnason. 1976. Cellular immunity in myasthenia gravis. Response to purified acetylcholine receptor and autologous thymocytes. *N Engl J Med 294:694*.
116. Wang, Z. Y., P. I. Karachunski, J. F. Howard, Jr., and B. M. Conti-Fine. 1999. Myasthenia in SCID mice grafted with myasthenic patient lymphocytes: role of CD4+ and CD8+ cells. *Neurology 52:484*.
117. Zhang, G. X., B. G. Xiao, M. Bakhiet, P. van der Meide, H. Wigzell, H. Link, and T. Olsson. 1996. Both CD4+ and CD8+ T cells are essential to induce experimental autoimmune myasthenia gravis. *J Exp Med 184:349*.
118. Ahlberg, R., Q. Yi, R. Pirskanen, G. Matell, C. Swerup, E. P. Rieber, G. Riethmuller, G. Holm, and A. K. Lefvert. 1994. Treatment of myasthenia gravis with anti-CD4 antibody: improvement correlates to decreased T-cell autoreactivity. *Neurology 44:1732*.
119. Sitaraman, S., D. W. Metzger, R. J. Belloto, A. J. Infante, and K. A. Wall. 2000. Interleukin-12 enhances clinical experimental autoimmune myasthenia gravis in susceptible but not resistant mice. *J Neuroimmunol 107:73*.
120. Karachunski, P. I., N. S. Ostlie, C. Monfardini, and B. M. Conti-Fine. 2000. Absence of IFN-gamma or IL-12 has different effects on experimental myasthenia gravis in C57BL/6 mice. *J Immunol 164:5236*.
121. Saoudi, A., I. Bernard, A. Hoedemaekers, B. Cautain, K. Martinez, P. Druet, M. De Baets, and J. C. Guery. 1999. Experimental autoimmune myasthenia gravis may occur in the context of a polarized Th1- or Th2-type immune response in rats. *J Immunol 162:7189*.
122. Im, S. H., D. Barchan, P. K. Maiti, S. Fuchs, and M. C. Souroujon. 2001. Blockade of CD40 ligand suppresses chronic experimental myasthenia gravis by down-regulation of Th1 differentiation and up-regulation of CTLA-4. *J Immunol 166:6893*.
123. Im, S. H., D. Barchan, P. K. Maiti, L. Raveh, M. C. Souroujon, and S. Fuchs. 2001. Suppression of experimental myasthenia gravis, a B cell-mediated autoimmune disease, by blockade of IL-18. *Faseb J 15:2140*.
124. Im, S. H., D. Barchan, S. Fuchs, and M. C. Souroujon. 1999. Suppression of ongoing experimental myasthenia by oral treatment with an acetylcholine receptor recombinant fragment. *J Clin Invest 104:1723*.
125. Karachunski, P. I., N. S. Ostlie, D. K. Okita, and B. M. Conti-Fine. 1999. Interleukin-4 deficiency facilitates development of experimental myasthenia gravis and precludes its prevention by nasal administration of CD4+ epitope sequences of the acetylcholine receptor. *J Neuroimmunol 95:73*.
126. Balasa, B., C. Deng, J. Lee, P. Christadoss, and N. Sarvetnick. 1998. The Th2 cytokine IL-4 is not required for the progression of antibody-dependent autoimmune myasthenia gravis. *J Immunol 161:2856*.
127. Zhang, G. X., B. G. Xiao, L. Y. Yu, P. H. van der Meide, and H. Link. 2001. Interleukin 10 aggravates experimental autoimmune myasthenia gravis through inducing Th2 and B cell responses to AChR. *J Neuroimmunol 113:10*.
128. Poussin, M. A., E. Goluszko, T. K. Hughes, S. I. Duchicella, and P. Christadoss. 2000. Suppression of experimental autoimmune myasthenia gravis in IL-10 gene-disrupted mice is associated with reduced B cells and serum cytotoxicity on mouse cell line expressing AChR. *J Neuroimmunol 111:152*.
129. Ostlie, N. S., P. I. Karachunski, W. Wang, C. Monfardini, M. Kronenberg, and B. M. Conti-Fine. 2001. Transgenic expression of IL-10 in T cells facilitates development of experimental myasthenia gravis. *J Immunol 166:4853*.
130. Huang, Y. M., P. Kivisakk, V. Ozenci, R. Pirskanen, and H. Link. 1999. Increased levels of circulating acetylcholine receptor (AChR)-reactive IL-10-secreting cells are characteristic for myasthenia gravis (MG). *Clin Exp Immunol 118:304*.

131. Yi, Q., R. Ahlberg, R. Pirskanen, and A. K. Lefvert. 1994. Acetylcholine receptor-reactive T cells in myasthenia gravis: evidence for the involvement of different subpopulations of T helper cells. *J Neuroimmunol 50:177*.
132. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol 155:1151*.
133. Jonuleit, H., E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk. 2001. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J Exp Med 193:1285*.
134. Seddon, B., and D. Mason. 2000. The third function of the thymus. *Immunol Today 21:95*.
135. Itoh, M., T. Takahashi, N. Sakaguchi, Y. Kuniyasu, J. Shimizu, F. Otsuka, and S. Sakaguchi. 1999. Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J Immunol 162:5317*.
136. Willerford, D. M., J. Chen, J. A. Ferry, L. Davidson, A. Ma, and F. W. Alt. 1995. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity 3:521*.
137. Luther, C., S. Poeschel, M. Varga, A. Melms, and E. Tolosa. 2005. Decreased frequency of intrathymic regulatory T cells in patients with myasthenia-associated thymoma. *J Neuroimmunol 164:124*.
138. Strobel, P., A. Rosenwald, N. Beyersdorf, T. Kerkau, O. Elert, A. Murumagi, N. Sillanpaa, P. Peterson, V. Hummel, P. Rieckmann, C. Burek, B. Schalke, W. Nix, R. Kiefer, H. K. Muller-Hermelink, and A. Marx. 2004. Selective loss of regulatory T cells in thymomas. *Ann Neurol 56:901*.
139. Balandina, A., S. Lecart, P. Dartevelle, A. Saoudi, and S. Berrih-Aknin. 2005. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood 105:735*.
140. Huang, Y. M., R. Pirskanen, R. Giscombe, H. Link, and A. K. Lefvert. 2004. Circulating CD4+CD25+ and CD4+CD25+ T cells in myasthenia gravis and in relation to thymectomy. *Scand J Immunol 59:408*.
141. Sun, Y., J. Qiao, C. Z. Lu, C. B. Zhao, X. M. Zhu, and B. G. Xiao. 2004. Increase of circulating CD4+CD25+ T cells in myasthenia gravis patients with stability and thymectomy. *Clin Immunol 112:284*.
142. Fattorossi, A., A. Battaglia, A. Buzzonetti, F. Ciaraffa, G. Scambia, and A. Evoli. 2005. Circulating and thymic CD4+CD25+ T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. *Immunology 116:134*.
143. Fontenot, J. D., and A. Y. Rudensky. 2005. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol 6:331*.
144. Bretscher, P., and M. Cohn. 1970. A theory of self-nonself discrimination. *Science 169:1042*.
145. Sperling, A. I., J. A. Auger, B. D. Ehst, I. C. Rulifson, C. B. Thompson, and J. A. Bluestone. 1996. CD28/B7 interactions deliver a unique signal to naive T cells that regulates cell survival but not early proliferation. *J Immunol 157:3909*.
146. Boise, L. H., A. J. Minn, P. J. Noel, C. H. June, M. A. Accavitti, T. Lindsten, and C. B. Thompson. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity 3:87*.
147. Lindstein, T., C. H. June, J. A. Ledbetter, G. Stella, and C. B. Thompson. 1989. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science 244:339*.
148. Fraser, J. D., B. A. Irving, G. R. Crabtree, and A. Weiss. 1991. Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. *Science 251:313*.

149. Appleman, L. J., A. Berezovskaya, I. Grass, and V. A. Boussiotis. 2000. CD28 costimulation mediates T cell expansion via IL-2-independent and IL-2-dependent regulation of cell cycle progression. *J Immunol 164:144*.
150. Boonen, G. J., A. M. van Dijk, L. F. Verdonck, R. A. van Lier, G. Rijksen, and R. H. Medema. 1999. CD28 induces cell cycle progression by IL-2-independent down-regulation of p27kip1 expression in human peripheral T lymphocytes. *Eur J Immunol 29:789*.
151. Brunner, M. C., C. A. Chambers, F. K. Chan, J. Hanke, A. Winoto, and J. P. Allison. 1999. CTLA-4-Mediated inhibition of early events of T cell proliferation. *J Immunol 162:5813*.
152. Greenwald, R. J., V. A. Boussiotis, R. B. Lorsbach, A. K. Abbas, and A. H. Sharpe. 2001. CTLA-4 regulates induction of anergy in vivo. *Immunity 14:145*.
153. Walunas, T. L., C. Y. Bakker, and J. A. Bluestone. 1996. CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med 183:2541*.
154. Hathcock, K. S., G. Laszlo, C. Pucillo, P. Linsley, and R. J. Hodes. 1994. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med 180:631*.
155. McLellan, A. D., G. C. Starling, L. A. Williams, B. D. Hock, and D. N. Hart. 1995. Activation of human peripheral blood dendritic cells induces the CD86 co-stimulatory molecule. *Eur J Immunol 25:2064*.
156. Fleischer, J., E. Soeth, N. Reiling, E. Grage-Griebenow, H. D. Flad, and M. Ernst. 1996. Differential expression and function of CD80 (B7-1) and CD86 (B7-2) on human peripheral blood monocytes. *Immunology 89:592*.
157. Caux, C., B. Vanbervliet, C. Massacrier, M. Azuma, K. Okumura, L. L. Lanier, and J. Banchereau. 1994. B70/B7-2 is identical to CD86 and is the major functional ligand for CD28 expressed on human dendritic cells. *J Exp Med 180:1841*.
158. Yokozeki, H., I. Katayama, O. Ohki, T. Matsunaga, K. Watanabe, T. Satoh, M. Azuma, K. Okumura, and K. Nishioka. 1996. Functional CD86 (B7-2/B70) on cultured human Langerhans cells. *J Invest Dermatol 106:147*.
159. Aruffo, A., and B. Seed. 1987. Molecular Cloning of a CD28 cDNA by a High-Efficiency COS Cell Expression System. *PNAS 84:8573*.
160. Azuma, M., H. Yssel, J. Phillips, H. Spits, and L. Lanier. 1993. Functional expression of B7/BB1 on activated T lymphocytes. *J. Exp. Med. 177:845*.
161. Harper, K., C. Balzano, E. Rouvier, M. Mattei, M. Luciani, and P. Golstein. 1991. CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *J Immunol 147:1037*.
162. Lindsten, T., K. Lee, E. Harris, B. Petryniak, N. Craighead, P. Reynolds, D. Lombard, G. Freeman, L. Nadler, and G. Gray. 1993. Characterization of CTLA-4 structure and expression on human T cells. *J Immunol 151:3489*.
163. Sabzevari, H., J. Kantor, A. Jaigirdar, Y. Tagaya, M. Naramura, J. Hodge, J. Bernon, and J. Schlom. 2001. Acquisition of CD80 (B7-1) by T cells. *J Immunol 166:2505*.
164. Tatari-Calderone, Z., R. T. Semnani, T. B. Nutman, J. Schlom, and H. Sabzevari. 2002. Acquisition of CD80 by human T cells at early stages of activation: functional involvement of CD80 acquisition in T cell to T cell interaction. *J Immunol 169:6162*.
165. Hollsberg, P., C. Scholz, D. E. Anderson, E. A. Greenfield, V. K. Kuchroo, G. J. Freeman, and D. A. Hafler. 1997. Expression of a hypoglycosylated form of CD86 (B7-2) on human T cells with altered binding properties to CD28 and CTLA-4. *J Immunol 159:4799*.
166. Jeannin, P., N. Herbault, Y. Delneste, G. Magistrelli, S. Lecoanet-Henchoz, G. Caron, J. P. Aubry, and J. Y. Bonnefoy. 1999. Human effector memory T cells express CD86: a functional role in naive T cell priming. *J Immunol 162:2044*.
167. van der Merwe, P. A., D. L. Bodian, S. Daenke, P. Linsley, and S. J. Davis. 1997. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med 185:393*.
168. Linsley, P. S., W. Brady, L. Grosmaire, A. Aruffo, N. K. Damle, and J. A. Ledbetter. 1991. Binding of the B cell activation antigen B7 to CD28

costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med 173:721*.
169. Linsley, P. S., W. Brady, M. Urnes, L. S. Grosmaire, N. K. Damle, and J. A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med 174:561*.
170. Linsley, P. S., J. L. Greene, W. Brady, J. Bajorath, J. A. Ledbetter, and R. Peach. 1994. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity 1:793*.
171. Linsley, P. S., J. Bradshaw, J. Greene, R. Peach, K. L. Bennett, and R. S. Mittler. 1996. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. *Immunity 4:535*.
172. Alegre, M. L., P. J. Noel, B. J. Eisfelder, E. Chuang, M. R. Clark, S. L. Reiner, and C. B. Thompson. 1996. Regulation of surface and intracellular expression of CTLA4 on mouse T cells. *J Immunol 157:4762*.
173. Vijayakrishnan, L., J. M. Slavik, Z. Illes, R. J. Greenwald, D. Rainbow, B. Greve, L. B. Peterson, D. A. Hafler, G. J. Freeman, A. H. Sharpe, L. S. Wicker, and V. K. Kuchroo. 2004. An autoimmune disease-associated CTLA-4 splice variant lacking the B7 binding domain signals negatively in T cells. *Immunity 20:563*.
174. Hanawa, H., Y. Ma, S. A. Mikolajczak, M. L. Charles, T. Yoshida, R. Yoshida, C. A. Strathdee, D. W. Litchfield, and A. Ochi. 2002. A novel costimulatory signaling in human T lymphocytes by a splice variant of CD28. *Blood 99:2138*.
175. Tivol, E. A., F. Borriello, A. N. Schweitzer, W. P. Lynch, J. A. Bluestone, and A. H. Sharpe. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity 3:541*.
176. Chambers, C. A., T. J. Sullivan, and J. P. Allison. 1997. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity 7:885*.
177. Tivol, E. A., S. D. Boyd, S. McKeon, F. Borriello, P. Nickerson, T. B. Strom, and A. H. Sharpe. 1997. CTLA4Ig prevents lymphoproliferation and fatal multiorgan tissue destruction in CTLA-4-deficient mice. *J Immunol 158:5091*.
178. Shahinian, A., K. Pfeffer, K. P. Lee, T. M. Kundig, K. Kishihara, A. Wakeham, K. Kawai, P. S. Ohashi, C. B. Thompson, and T. W. Mak. 1993. Differential T cell costimulatory requirements in CD28-deficient mice. *Science 261:609*.
179. Green, J. M., P. J. Noel, A. I. Sperling, T. L. Walunas, G. S. Gray, J. A. Bluestone, and C. B. Thompson. 1994. Absence of B7-dependent responses in CD28-deficient mice. *Immunity 1:501*.
180. Borriello, F., M. P. Sethna, S. D. Boyd, A. N. Schweitzer, E. A. Tivol, D. Jacoby, T. B. Strom, E. M. Simpson, G. J. Freeman, and A. H. Sharpe. 1997. B7-1 and B7-2 have overlapping, critical roles in immunoglobulin class switching and germinal center formation. *Immunity 6:303*.
181. Gough, S. C., L. S. Walker, and D. M. Sansom. 2005. CTLA4 gene polymorphism and autoimmunity. *Immunol Rev 204:102*.
182. Jeannin, P., G. Magistrelli, J. P. Aubry, G. Caron, J. F. Gauchat, T. Renno, N. Herbault, L. Goetsch, A. Blaecke, P. Y. Dietrich, J. Y. Bonnefoy, and Y. Delneste. 2000. Soluble CD86 is a costimulatory molecule for human T lymphocytes. *Immunity 13:303*.
183. Hock, B., G. Starling, W. Patton, N. Salm, K. Bond, L. McArthur, and J. McKenzie. 2004. Identification of a Circulating Soluble Form of CD80: Levels in Patients with Hematological Malignancies. *Leuk Lymphoma 45:2111*.
184. Hebbar, M., P. Jeannin, G. Magistrelli, P. Y. Hatron, E. Hachulla, B. Devulder, J. Y. Bonnefoy, and Y. Delneste. 2004. Detection of circulating soluble CD28 in patients with systemic lupus erythematosus, primary Sjogren's syndrome and systemic sclerosis. *Clin Exp Immunol 136:388*.
185. Oaks, M. K., K. M. Hallett, R. T. Penwell, E. C. Stauber, S. J. Warren, and A. J. Tector. 2000. A native soluble form of CTLA-4. *Cell Immunol 201:144*.
186. Wong, C. K., L. C. Lit, L. S. Tam, E. K. Li, and C. W. Lam. 2005. Aberrant production of soluble costimulatory molecules CTLA-4, CD28, CD80 and CD86 in patients with systemic lupus erythematosus. *Rheumatology (Oxford)*.

187. Ip, W. K., C. K. Wong, T. F. Leung, and C. W. Lam. 2005. Elevation of plasma soluble T cell costimulatory molecules CTLA-4, CD28 and CD80 in children with allergic asthma. *Int Arch Allergy Immunol 137:45*.
188. Oaks, M. K., and K. M. Hallett. 2000. Cutting edge: a soluble form of CTLA-4 in patients with autoimmune thyroid disease. *J Immunol 164:5015*.
189. Liu, M. F., C. R. Wang, P. C. Chen, and L. L. Fung. 2003. Increased expression of soluble cytotoxic T-lymphocyte-associated antigen-4 molecule in patients with systemic lupus erythematosus. *Scand J Immunol 57:568*.
190. Sato, S., M. Fujimoto, M. Hasegawa, K. Komura, K. Yanaba, I. Hayakawa, T. Matsushita, and K. Takehara. 2004. Serum soluble CTLA-4 levels are increased in diffuse cutaneous systemic sclerosis. *Rheumatology (Oxford) 43:1261*.
191. Wong, C. K., S. W. Lun, F. W. Ko, W. K. Ip, D. S. Hui, and C. W. Lam. 2005. Increased expression of plasma and cell surface co-stimulatory molecules CTLA-4, CD28 and CD86 in adult patients with allergic asthma. *Clin Exp Immunol 141:122*.
192. Hock, B. D., W. N. Patton, S. Budhia, D. Mannari, P. Roberts, and J. L. McKenzie. 2002. Human plasma contains a soluble form of CD86 which is present at elevated levels in some leukaemia patients. *Leukemia 16:865*.
193. Shi, H. Z., Z. F. Xie, J. M. Deng, Y. Q. Chen, and C. Q. Xiao. 2004. Soluble CD86 protein in serum samples of patients with asthma. *Thorax 59:870*.
194. Kremer, J. M., R. Westhovens, M. Leon, E. Di Giorgio, R. Alten, S. Steinfeld, A. Russell, M. Dougados, P. Emery, I. F. Nuamah, G. R. Williams, J. C. Becker, D. T. Hagerty, and L. W. Moreland. 2003. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med 349:1907*.
195. Linsley, P. S., P. M. Wallace, J. Johnson, M. G. Gibson, J. L. Greene, J. A. Ledbetter, C. Singh, and M. A. Tepper. 1992. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. *Science 257:792*.
196. Yamaguchi, N., S. Hiraoka, T. Mukai, N. Takeuchi, X. Y. Zhou, S. Ono, M. Kogo, K. Dunussi-Joannopoulos, V. Ling, S. Wolf, and H. Fujiwara. 2004. Induction of tumor regression by administration of B7-Ig fusion proteins: mediation by type 2 CD8+ T cells and dependence on IL-4 production. *J Immunol 172:1347*.
197. Todo, T., R. L. Martuza, M. J. Dallman, and S. D. Rabkin. 2001. In situ expression of soluble B7-1 in the context of oncolytic herpes simplex virus induces potent antitumor immunity. *Cancer Res 61:153*.
198. Sturmhoefel, K., K. Lee, G. S. Gray, J. Thomas, R. Zollner, M. O'Toole, H. Swiniarski, A. Dorner, and S. F. Wolf. 1999. Potent activity of soluble B7-IgG fusion proteins in therapy of established tumors and as vaccine adjuvant. *Cancer Res 59:4964*.
199. Flo, J., S. Tisminetzky, and F. Baralle. 2001. Codelivery of DNA coding for the soluble form of CD86 results in the down-regulation of the immune response to DNA vaccines. *Cell Immunol 209:120*.
200. Chen, A., H. J. Meyerson, A. Salvekar, and M. L. Tykocinski. 1998. Non-glycosylated human B7-1(CD80) retains the capacity to bind its counter-receptors. *FEBS Lett 428:127*.
201. Broeren, C. P., G. S. Gray, B. M. Carreno, and C. H. June. 2000. Costimulation light: activation of CD4+ T cells with CD80 or CD86 rather than anti-CD28 leads to a Th2 cytokine profile. *J Immunol 165:6908*.
202. Gerstmayer, B., U. Pessara, and W. Wels. 1997. Construction and expression in the yeast Pichia pastoris of functionally active soluble forms of the human costimulatory molecules B7-1 and B7-2 and the B7 counter-receptor CTLA-4. *FEBS Lett 407:63*.
203. Shen, W., Y. Wang, Y. Geng, and L. Si. 2000. Bacterially produced human B7-1 protein encompassing its complete extracellular domain maintains its costimulatory activity in vitro. *Chin Med J (Engl) 113:714*.
204. Faas, S. J., M. A. Giannoni, A. P. Mickle, C. L. Kiesecker, D. J. Reed, D. Wu, W. L. Fodor, J. P. Mueller, L. A. Matis, and R. P. Rother. 2000. Primary structure and functional characterization of a soluble, alternatively spliced form of B7-1. *J Immunol 164:6340*.

205. Grohmann, U., C. Orabona, F. Fallarino, C. Vacca, F. Calcinaro, A. Falorni, P. Candeloro, M. L. Belladonna, R. Bianchi, M. C. Fioretti, and P. Puccetti. 2002. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol 3:1097*.
206. Orabona, C., U. Grohmann, M. L. Belladonna, F. Fallarino, C. Vacca, R. Bianchi, S. Bozza, C. Volpi, B. L. Salomon, M. C. Fioretti, L. Romani, and P. Puccetti. 2004. CD28 induces immunostimulatory signals in dendritic cells via CD80 and CD86. *Nat Immunol 5:1134*.
207. Kroczek, R. A., H. W. Mages, and A. Hutloff. 2004. Emerging paradigms of T-cell co-stimulation. *Curr Opin Immunol 16:321*.
208. Somoza, C., and L. L. Lanier. 1995. T-cell costimulation via CD28-CD80/CD86 and CD40-CD40 ligand interactions. *Res Immunol 146:171*.
209. Poussin, M. A., E. Tuzun, E. Goluszko, B. G. Scott, H. Yang, J. U. Franco, and P. Christadoss. 2003. B7-1 costimulatory molecule is critical for the development of experimental autoimmune myasthenia gravis. *J Immunol 170:4389*.
210. Scott, B. G., H. Yang, E. Tuzun, C. Dong, R. A. Flavell, and P. Christadoss. 2004. ICOS is essential for the development of experimental autoimmune myasthenia gravis. *J Neuroimmunol 153:16*.
211. Shi, F. D., B. He, H. Li, D. Matusevicius, H. Link, and H. G. Ljunggren. 1998. Differential requirements for CD28 and CD40 ligand in the induction of experimental autoimmune myasthenia gravis. *Eur J Immunol 28:3587*.
212. Wang, H. B., F. D. Shi, H. Li, B. J. Chambers, H. Link, and H. G. Ljunggren. 2001. Anti-CTLA-4 antibody treatment triggers determinant spreading and enhances murine myasthenia gravis. *J Immunol 166:6430*.
213. McIntosh, K. R., P. S. Linsley, P. A. Bacha, and D. B. Drachman. 1998. Immunotherapy of experimental autoimmune myasthenia gravis: selective effects of CTLA4Ig and synergistic combination with an IL2-diphtheria toxin fusion protein. *J Neuroimmunol 87:136*.
214. Teleshova, N., D. Matusevicius, P. Kivisakk, M. Mustafa, R. Pirskanen, and H. Link. 2000. Altered expression of costimulatory molecules in myasthenia gravis. *Muscle Nerve 23:946*.
215. Franciotta, D., G. Martino, E. Brambilla, E. Zardini, V. Locatelli, A. Bergami, C. Tinelli, G. Desina, and V. Cosi. 1999. TE671 cell-based ELISA for anti-acetylcholine receptor antibody determination in myasthenia gravis. *Clin Chem 45:400*.
216. Somnier, F. E. 1994. Anti-acetylcholine receptor (AChR) antibodies measurement in myasthenia gravis: the use of cell line TE671 as a source of AChR antigen. *J Neuroimmunol 51:63*.
217. Fingeroth, J. D., J. J. Weis, T. F. Tedder, J. L. Strominger, P. A. Biro, and D. T. Fearon. 1984. Epstein--Barr Virus Receptor of Human B Lymphocytes is the C3d Receptor CR2. *PNAS 81:4510*.
218. Roome, A. J., and C. L. Reading. 1987. Frequency of B-lymphocyte transformation by Epstein-Barr virus decreases with entry into the cell cycle. *Immunology 60:195*.
219. Aman, P., B. Ehlin-Henriksson, and G. Klein. 1984. Epstein-Barr virus susceptibility of normal human B lymphocyte populations. *J Exp Med 159:208*.
220. Boyd, A. W., K. C. Anderson, A. S. Freedman, D. C. Fisher, B. Slaughenhoupt, S. F. Schlossman, and L. M. Nadler. 1985. Studies of in vitro activation and differentiation of human B lymphocytes. I. Phenotypic and functional characterization of the B cell population responding to anti-Ig antibody. *J Immunol 134:1516*.
221. Tuzun, E., B. G. Scott, E. Goluszko, S. Higgs, and P. Christadoss. 2003. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. *J Immunol 171:3847*.
222. Christadoss, P. 1988. C5 gene influences the development of murine myasthenia gravis. *J Immunol 140:2589*.
223. Lin, F., H. J. Kaminski, B. M. Conti-Fine, W. Wang, C. Richmonds, and M. E. Medof. 2002. Markedly enhanced susceptibility to experimental autoimmune

myasthenia gravis in the absence of decay-accelerating factor protection. *J Clin Invest 110:1269*.
224. Selmi, C., M. J. Mayo, N. Bach, H. Ishibashi, P. Invernizzi, R. G. Gish, S. C. Gordon, H. I. Wright, B. Zweiban, M. Podda, and M. E. Gershwin. 2004. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. *Gastroenterology 127:485*.
225. Nelson, J. L. 2002. Microchimerism: incidental byproduct of pregnancy or active participant in human health? *Trends Mol Med 8:109*.
226. Invernizzi, P., M. Miozzo, P. M. Battezzati, I. Bianchi, F. R. Grati, G. Simoni, C. Selmi, M. Watnik, M. E. Gershwin, and M. Podda. 2004. Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet 363:533*.
227. Kahan, B. D., P. R. Rajagopalan, and M. Hall. 1999. Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a chimeric anti-interleukin-2-receptor monoclonal antibody. United States Simulect Renal Study Group. *Transplantation 67:276*.
228. McHugh, R. S., and E. M. Shevach. 2002. Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol 168:5979*.
229. Ueda, H., J. M. Howson, L. Esposito, J. Heward, H. Snook, G. Chamberlain, D. B. Rainbow, K. M. Hunter, A. N. Smith, G. Di Genova, M. H. Herr, I. Dahlman, F. Payne, D. Smyth, C. Lowe, R. C. Twells, S. Howlett, B. Healy, S. Nutland, H. E. Rance, V. Everett, L. J. Smink, A. C. Lam, H. J. Cordell, N. M. Walker, C. Bordin, J. Hulme, C. Motzo, F. Cucca, J. F. Hess, M. L. Metzker, J. Rogers, S. Gregory, A. Allahabadia, R. Nithiyananthan, E. Tuomilehto-Wolf, J. Tuomilehto, P. Bingley, K. M. Gillespie, D. E. Undlien, K. S. Ronningen, C. Guja, C. Ionescu-Tirgoviste, D. A. Savage, A. P. Maxwell, D. J. Carson, C. C. Patterson, J. A. Franklyn, D. G. Clayton, L. B. Peterson, L. S. Wicker, J. A. Todd, and S. C. Gough. 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature 423:506*.
230. Huang, D., R. Giscombe, Y. Zhou, R. Pirskanen, and A. K. Lefvert. 2000. Dinucleotide repeat expansion in the CTLA-4 gene leads to T cell hyper-reactivity via the CD28 pathway in myasthenia gravis. *J Neuroimmunol 105:69*.
231. Takara, M., T. Kouki, and L. J. DeGroot. 2003. CTLA-4 AT-repeat polymorphism reduces the inhibitory function of CTLA-4 in Graves' disease. *Thyroid 13:1083*.
232. Kouki, T., Y. Sawai, C. A. Gardine, M. E. Fisfalen, M. L. Alegre, and L. J. DeGroot. 2000. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol 165:6606*.
233. Lum, L. G., N. Orcutt-Thordarson, M. C. Seigneuret, and J. A. Hansen. 1982. In vitro regulation of immunoglobulin synthesis by T-cell subpopulations defined by a new human T-cell antigen (9.3). *Cell Immunol 72:122*.
234. Filaci, G., M. Fravega, S. Negrini, F. Procopio, D. Fenoglio, M. Rizzi, S. Brenci, P. Contini, D. Olive, M. Ghio, M. Setti, R. S. Accolla, F. Puppo, and F. Indiveri. 2004. Nonantigen specific CD8+ T suppressor lymphocytes originate from CD8+CD28- T cells and inhibit both T-cell proliferation and CTL function. *Hum Immunol 65:142*.
235. Filaci, G., M. Fravega, D. Fenoglio, M. Rizzi, S. Negrini, R. Viggiani, and F. Indiveri. 2004. Non-antigen specific CD8+ T suppressor lymphocytes. *Clin Exp Med 4:86*.
236. Effros, R. B. 1997. Loss of CD28 expression on T lymphocytes: a marker of replicative senescence. *Dev Comp Immunol 21:471*.
237. Vallejo, A. N., C. M. Weyand, and J. J. Goronzy. 2004. T-cell senescence: a culprit of immune abnormalities in chronic inflammation and persistent infection. *Trends Mol Med 10:119*.
238. Wang, X. B., X. Zhao, R. Giscombe, and A. K. Lefvert. 2002. A CTLA-4 gene polymorphism at position -318 in the promoter region affects the expression of protein. *Genes Immun 3:233*.
239. Oosterhuis, H. J. 1984. *Myasthenia gravis*. Churchill Livingstone, Edinburgh.

240. Steiner, K., I. Waase, T. Rau, M. Dietrich, B. Fleischer, and B. M. Broker. 1999. Enhanced expression of CTLA-4 (CD152) on CD4+ T cells in HIV infection. *Clin Exp Immunol 115:451*.
241. Steiner, K., F. Moosig, E. Csernok, K. Selleng, W. L. Gross, B. Fleischer, and B. M. Broker. 2001. Increased expression of CTLA-4 (CD152) by T and B lymphocytes in Wegener's granulomatosis. *Clin Exp Immunol 126:143*.
242. Brown, R. D., B. Pope, E. Yuen, J. Gibson, and D. E. Joshua. 1998. The expression of T cell related costimulatory molecules in multiple myeloma. *Leuk Lymphoma 31:379*.
243. Frydecka, I., A. Kosmaczewska, D. Bocko, L. Ciszak, D. Wolowiec, K. Kuliczkowski, and I. Kochanowska. 2004. Alterations of the expression of T-cell-related costimulatory CD28 and downregulatory CD152 (CTLA-4) molecules in patients with B-cell chronic lymphocytic leukaemia. *Br J Cancer 90:2042*.
244. Liu, M. F., C. Y. Yang, J. S. Li, K. A. Lai, S. C. Chao, and H. Y. Lei. 1999. Increased expression of down-regulatory CTLA-4 molecule on T lymphocytes from rheumatoid synovial compartment. *Scand J Immunol 50:68*.
245. Matsubara, T., R. Anwar, M. Fujiwara, T. Ichiyama, and S. Furukawa. 2003. CTLA-4 (CD152) expression in peripheral blood T cells in Kawasaki disease. *Clin Exp Immunol 132:169*.
246. Liu, M. F., H. S. Liu, C. R. Wang, and H. Y. Lei. 1998. Expression of CTLA-4 molecule in peripheral blood T lymphocytes from patients with systemic lupus erythematosus. *J Clin Immunol 18:392*.
247. Schlotmann, T., I. Waase, C. Julch, U. Klauenberg, B. Muller-Myhsok, M. Dietrich, B. Fleischer, and B. M. Broker. 2000. CD4 alphabeta T lymphocytes express high levels of the T lymphocyte antigen CTLA-4 (CD152) in acute malaria. *J Infect Dis 182:367*.
248. Paas-Rozner, M., M. Sela, and E. Mozes. 2003. A dual altered peptide ligand down-regulates myasthenogenic T cell responses by up-regulating CD25- and CTLA-4-expressing CD4+ T cells. *Proc Natl Acad Sci U S A 100:6676*.
249. Tesar, V., E. Jelinkova, M. Jirsa, Jr., M. Bakosova, P. Pitha, and V. Chabova. 2000. Soluble adhesion molecules and cytokines in patients with myasthenia gravis treated by plasma exchange. *Blood Purif 18:115*.
250. Confalonieri, P., C. Antozzi, F. Cornelio, O. Simoncini, and R. Mantegazza. 1993. Immune activation in myasthenia gravis: soluble interleukin-2 receptor, interferon-gamma and tumor necrosis factor-alpha levels in patients' serum. *J Neuroimmunol 48:33*.
251. Cohen-Kaminsky, S., Y. Jacques, C. Aime, D. Safar, E. Morel, and S. Berrih-Aknin. 1992. Follow-up of soluble interleukin-2 receptor levels after thymectomy in patients with myasthenia gravis. *Clin Immunol Immunopathol 62:190*.
252. Magistrelli, G., P. Jeannin, N. Herbault, A. Benoit De Coignac, J. F. Gauchat, J. Y. Bonnefoy, and Y. Delneste. 1999. A soluble form of CTLA-4 generated by alternative splicing is expressed by nonstimulated human T cells. *Eur J Immunol 29:3596*.
253. Magistrelli, G., P. Jeannin, G. Elson, J. F. Gauchat, T. N. Nguyen, J. Y. Bonnefoy, and Y. Delneste. 1999. Identification of three alternatively spliced variants of human CD28 mRNA. *Biochem Biophys Res Commun 259:34*.
254. Ruderman, E. M., and R. M. Pope. 2005. The evolving clinical profile of abatacept (CTLA4-Ig): a novel co-stimulatory modulator for the treatment of rheumatoid arthritis. *Arthritis Res Ther 7 Suppl 2:S21*.