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# Immune Dysregulation in Myelodysplastic Syndromes: Pathogenetic-Pathophysiologic Aspects and Clinical Consequences

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Additional information is available at the end of the chapter

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## Abstract

Myelodysplastic syndromes are clonal hematopoietic stem cell disorders, in which the immune system plays a substantial pathogenetic role. Patients manifest frequent infections, mainly attributed to neutropenia, but sometimes opportunistic pathogens are isolated in non-neutropenic patients. They also exhibit autoimmune diseases or syndromes with a background of immune activation and various “abnormalities” of T-lymphocytes, B-lymphocytes, and NK cells. The most typical profile includes reduced total T lymphocytes (mainly CD4+ helper T-cells, resulting in decrease or inversion of the CD4/CD8 cell ratio) and impaired NK cell function. Many TH1 direction cytokines, and particularly sIL-2R, IL-6, and TNF- $\alpha$  are usually found increased in the serum and bone marrow, which have been strongly associated with advanced disease, anemia, and other disease-related features. Clonal origin of lymphocytes has been confirmed only in few cases. Mixed lymphocyte cultures and genomic assays have shown severely impaired immunoregulatory abnormalities, probably induced by the hematopoietic cells. In a minority of patients, immune activation is capable to prevent or delay clonal expansion, but these patients have more profound hematopoietic impairment. Immunosuppressive treatment may not only relieve the autoimmune manifestations but also improve hematopoiesis. However, this kind of treatment is not well tolerated, is associated with severe infections, and in some cases may enhance AML evolution.

**Keywords:** myelodysplastic syndromes, pathogenesis, immune abnormalities, autoimmune diseases, immunosuppressive treatment

## 1. Introduction

Myelodysplastic syndromes (MDS) are diseases emerging from somatic mutations of a pluripotent hematopoietic stem cell, affecting its functional capacity for maturation and differentiation, but preserving it alive and capable to escape from apoptotic signals. The affected cell creates a clone, gradually suppressing nonclonal cells, and finally dominating in the bone marrow. Clonal cells are prone to additional genetic events, promoting survival advantage and further impairing differentiation and maturation, thus generating a neoplastic phenotype, with the evolution to acute myelogenous leukemia (AML). Since such genetic events occur serially, but with a variable evolutionary potential, MDS represent probably the best *in vivo* model of “step-by-step” progression from a premalignant state to a high-potential neoplasm, such as AML. The multiparametric study of MDS can draw messages and conclusions, potentially applicable for the pathogenesis and pathophysiology of all types of neoplasia.

One rather unexpected aspect of MDS pathophysiology is the strong involvement of the immune system. Soon after the initial description of MDS, many “immune abnormalities” were reported in the literature. The “easy” initial interpretation of the participation of the immune system to the dysplastic clone was quickly changed, in favor of other explanations. But even now, a clear interpretation and a definite treatment strategy for these “abnormalities” have not been established. In this chapter, we describe the spectrum of “immune abnormalities” of MDS and briefly discuss treatment approaches, targeting the immune system. Besides a thorough literature review, we have used our personal experience, based on the study of more than 1500 patients. Thus, this chapter includes also original data, presented at various meetings, but not yet published as full papers, emerged from personal/institutional research activities at the Department of Hematology of the University Hospital of Patras.

## 2. Infections unrelated to the severity of neutropenia among patients with MDS

Infections of various etiologies are common among MDS patients and represent one of the major presenting features, but also a leading cause of morbidity and mortality. Beside the usually advanced patient age and comorbidity, the major predisposing factor in many retrospective studies analyzing the frequency and severity of infections is the depth of neutropenia. Functional neutrophil abnormalities have also been reported, such as impaired locomotion and chemotaxis, reduced complement receptor-1 and -3 expressions, and reduced enzymatic armamentarium, resulting in impaired respiratory burst and reduced bactericidal and fungicidal capacity. These defects have been observed in all types of MDS but are more frequent in the higher risk groups [1]. However, severe common and opportunistic infections may be manifested in nonneutropenic patients. In these cases, besides the functional neutrophil impairment, various acquired defects of the adaptive immunity, affecting immunocompetent cell populations have been proposed as predisposing factors. Finally, transfusions, transfusion-induced iron overload, and the newer treatment modalities, such as lenalidomide and

hypomethylating agents, may hamper immune functions and contribute to the development of infections.

There are several published cases or small series of patients manifesting pyogenic collections/abscesses, not only at common sites (perianal, splenic, liver), but also at rare and uncommon (pararenal, intramuscular, paracolic, etc.), without the development of strong inflammatory reaction [2, 3]. It has been suggested that mature granulocytes of MDS patients may not produce effective inflammatory reaction to eliminate pathogens, and induce the formation of granulomas or abscesses [1, 2]. MDS patients, even when nonneutropenic, may exhibit delayed healing of infections and have increased intracellular neutrophil collagenase activity, irrespective of WHO or IPSS subgroup [3].

Other patients manifest bacterial, viral, and fungal infections, from rare/opportunistic pathogens, before any immunosuppressive or cytotoxic treatment, similar to those encountered among patients with underlying congenital or acquired immunodeficiency. Among such rare bacterial pathogens, coagulase-negative *Staphylococci*, rare Enterococci, *Myc. avium-intracellulare*, *Myc. Kansasii*, *Myc. Malmöense*, *Bacillus cereus*, *Corynebacterium* and *Phenyllobacterium* spp., *Aeromonas hydrophila*, *Brevundimonas diminuta*, *Rhodococcus corynebacterioides*, and *Bordetella hinzii* are included. Among viral, fungal, and other pathogens, CMV and EBV reactivation, HHV-6 infection, JCV-induced progressive multifocal leukoencephalopathy (PML), *Pneumocystis carinii*, and *Legionella pneumophila* are included. Invasive fungal infections are rather uncommon and may emerge during the myelosuppression, which follows cytotoxic chemotherapy and/or allogeneic stem cell transplantation, in patients receiving prophylactically or therapeutically strong combinations of antibacterial antibiotics. However, many cases have been reported in the absence of these recognized predisposing factors. Involved pathogens are both, yeasts, including *C. albicans*, *Candida non-albicans* spp., and *Cryptococcus neoformans* and molds, such as *Aspergillus* and zygomycetes, and the usually affected organs are the lungs, liver, spleen, and central nervous system (CNS), and more rarely the skin, soft tissues, and other organs.

### **3. Association of autoimmune and immune-mediated diseases, with the manifestation of MDS**

#### **3.1. Clinical syndromes of immune overactivity associated with myelodysplastic syndromes**

##### *3.1.1. Clearly autoimmune diseases*

Only few years after the recognition of MDS as separate entities and the proposal of their first classification system (FAB classification), it was obvious that they were associated with increased frequency of various immune abnormalities, either abnormal laboratory findings, such as organ- and non-organ-specific autoantibodies, or true clinical syndromes or diseases, reflecting severely impaired adaptive immunity.

Among the autoimmune or immune-mediated clinical syndromes, described in association with MDS, Coombs-positive immunohemolytic anemia (AIHA) [4], immune thrombocytope-

nia (ITP) [5], Evans' syndrome, autoimmune neutropenia, and pure red cell aplasia (usually drug-related) are included. AIHA is somewhat more common, and is usually of warm type, associated with mild to moderate chronic hemolysis [4]. It can be automatically presented or triggered by red blood cell transfusions. Treatment is mainly based on corticosteroids, but response rate is lower than in the idiopathic cases, and even when a "complete" response is achieved, this cannot be objectively evaluated due to coexistence of the basic disease, which has as a major manifestation anemia, although not clearly hemolytic. When corticosteroids are ineffective or associated with unacceptable toxicity, cyclosporine-A, mofetil mycophenolate, or pulses of vinca alkaloids may be used. In some instances immunosuppressive treatment may be accompanied by trilineage response, improving also neutrophil and platelet counts.

Immune thrombocytopenia particularly of chronic type, when manifested in elderly patients may mimic true MDS, particularly when there is additional underlying comorbidity and patients also have anemia of chronic disease. In such instances, the differential diagnosis is difficult and this is clearly an overlapping area of hematological disorders [6]. More complicating is the fact that these two different entities may share some common pathogenetic features, concerning premature megakaryocyte cell death [7]. However, true immune thrombocytopenia with high titers of antiplatelet autoantibodies may be the presenting feature [6, 8] or may complicate the course of a previously diagnosed MDS [9]. In some instances, ITP may precede and MDS may follow some months or years, even after the achievement of complete response of the ITP. Thrombocytopenia has been related to higher amount of glycolalycin and platelet-associated IgG, higher MPV, more advanced disease, and worse prognosis in patients with MDS. The occurrence of ITP has been more frequently reported in chronic myelomonocytic leukemia (CMML) and the del-5q syndrome, whereas relatively severe thrombocytopenia, with mild-moderate or absence of anemia and neutropenia has been reported among patients with isolated del-20q [10]. Retrospective evaluation of 123 patients with CMML revealed the presence of auto-/hyperimmune disorders in 19.5% of them, compared to 3–4% incidence in the general population [11]. CMML has been considered the MDS, most frequently associated with "paraneoplastic" manifestations. Finally, high frequency of hypocomplementemia, often associated with severe cytopenia, particularly in patients with lower risk MDS has been reported, suggesting the possible contribution of autoimmune mechanisms in its pathogenesis [12].

### *3.1.2. Common clinical syndromes with a dominantly immune pathogenesis*

Dominant position among the immune hyperactivity/autoimmunity syndromes possess the various systemic vasculitides, such as febrile neutrophilic dermatosis (Sweet's syndrome) [13], other leucocytoclastic vasculitides, and necrotizing panniculitis, most commonly localized in the skin and accompanied by rashes or resulting in extended skin ulcerations. Large vessel arteritis (Takayasu's disease), aortitis, and other organ-specific vasculitides, such as Wegener's granulomatosis, have been reported. Additional cutaneous manifestations associated with the occult or prominent presence of an MDS include granulomatous eruptions, pyoderma gangrenosum, erythema nodosum, erythema elevatum diutinum, bullous pemphigoid, cutaneous lupus, Behçet's disease, dermatomyositis, and Raynaud's syndrome.

Hematological	(Other) Cutaneous syndromes
Immunohemolytic anemia	Erythema nodosum
Immune thrombocytopenia	Pyoderma gangrenosum
Evans' syndrome	Erythema elevatum diutinum
Chronic cold agglutinin disease	Bullous pemphigoid
Autoimmune neutropenia	Cutaneous lupus
Pure red cell aplasia	Granulomatous eruptions
	Raynaud phenomenon
<b>Connective tissue type</b>	<b>Other syndromes</b>
Systemic lupus erythematosus	Fever of unknown origin (disease related)
Classical seropositive rheumatoid arthritis	Sarcoidosis – sarcoidotic-type granulomata
Seronegative rheumatoid arthritis	Iridocyclitis – uveitis
Sjögren's syndrome	Sterile osteomyelitis
Mixed connective tissue disease	Hashimoto's thyroiditis
Polymyalgia rheumatica	Addison disease
Seronegative migratory synovitis	Behcet disease
Remitting symmetrical synovitis with pitting edema	Pericarditis
Eosinophilic fasciitis	Pleural effusions
Relapsing polychondritis	Chronic autoimmune hepatitis
Polymyosistis	Inflammatory bowel disease
Dematomyosistis	Ulcerative colitis
<b>Vasculitic type</b>	Autoimmune pancreatitis
Sweet's syndrome	Pulmonary alveolar proteinosis
Leucocytoclastic vasculitis	Bronchiolitis obliterans organizing pneumonia
Periarteritis nodosa	Nephrotic syndrome
Wegener granulomatosis	Segmental glomerulosclerosis
Large vessel arteritis (Takayasu disease)	Peripheral demyelinating polyneuropathy
Aortitis	Expressive aphasia
Necrotizing panniculitis	

**Table 1.** Clinical syndromes of auto-/hyperimmune basis associated with myelodysplasia.

Fever of unknown origin in the absence of any known underlying condition has been described in association with MDS and may be accompanied by mild lymphadenopathy and sarcoidic-type noncaseating granulomata, high serum ferritin, and polyclonal hyper- $\gamma$ -globulinemia.



Various rheumatic manifestations may also be associated with an MDS and some patients are diagnosed following an initial presentation of a typical rheumatic disease. Remitting seronegative symmetrical synovitis with pitting edema has been reported as an initial presentation of MDS, with subsequent manifestation of relapsing polychondritis. Besides the more frequent than expected classical rheumatoid arthritis and systemic lupus erythematosus, seronegative migratory synovitis, various seropositive and seronegative polyarthritic syndromes, polymyositis, polymyalgia rheumatica, eosinophilic fasciitis, Sjögren's syndrome, and mixed connective tissue disease have also been reported.

Less common syndromes are noninfectious serosal effusions, usually pleural, but sometimes also pericarditis, chronic autoimmune hepatitis, Hashimoto's thyroiditis, Addison's disease, inflammatory bowel disease, glomerulonephritis and nephrotic syndrome, focal and segmental glomerulosclerosis, chronic autoimmune pancreatitis, ulcerative colitis, and various syndromes reflecting immune-based inflammatory processes of the CNS, such as seizures, expressive aphasia and paresis, and peripheral demyelinating polyneuropathy [14]. Finally, noninfectious pulmonary infiltrates, sometimes typical for alveolar proteinosis and bronchiolitis obliterans organizing pneumonia (BOOP), in the absence of previous allogeneic transplantation have also been reported. **Table 1** summarizes the various auto-/hyperimmune syndromes, sometimes called "paraneoplastic," associated with MDS.

### 3.1.3. *Relapsing polychondritis*

Of particular interest is the syndrome of relapsing polychondritis, which, besides the presentation as an idiopathic autoimmune syndrome, has almost exclusively been reported in association with MDS and very rarely with other diseases. Thus, among newly diagnosed polychondritis, without any evidence for a hematological disorder, BM examination may reveal the presence of an as yet undiagnosed MDS [15]. Polychondritis is manifested as painful inflammation of the cartilaginous areas of the body, such as the external ear, the basal area of the nose and the nasal septum, the synovial cartilage, and the tracheal and bronchial cartilaginous rings. Symptomatic period may persist for many days or some weeks, followed by resolution, but symptoms reappear after weeks or months. The cartilage is finally destroyed, resulting in anatomical malformation and functional disturbances. The syndrome may be associated with fever, renal, cardiovascular, or ocular manifestations, as well as by symptoms, related to organ-specific dysfunction, not clearly containing cartilaginous tissue [16]. Pathogenesis is clearly immune-based and may reflect immunological reaction against some marrow stromal elements, which also exist in the cartilaginous tissue. Prompt intervention with corticosteroids accelerates resolution of the inflammatory reaction and may reduce tissue destruction and malformations.

### 3.1.4. *Overview of patient series and epidemiological data on autoimmune manifestations*

Autoimmune diseases have been reported on average in 10–30% of MDS patients, in all age groups and disease subtypes sometimes more frequently among females and in patients with higher risk MDS or CMML [9, 17]. In some early studies, the frequency of true autoimmune diseases among series of MDS patients was not found increased compared to non-MDS

subjects of similar age. Moreover, the detection of various autoantibodies, directed against erythrocytic, neutrophilic, and platelet components, in the serum of MDS patients has been disputed, whether it really represents an immune abnormality, and has been attributed to the advanced patient age to thorough searching processes or to alloimmunization from the frequent transfusions. It has been suggested, although not proved, that similar results could be obtained from multiply transfused non-MDS patients of advanced age [18]. In another study, patients exhibiting immune abnormalities were younger and had mainly therapy-related MDS with complex chromosomal aberrations [9]. Among the reported cases with available cytogenetics, trisomy 8 cases are rather overrepresented, but in the majority of described series of patients no clear preponderance of any demographic, cytogenetic, or histological feature was found [19]. In some studies, the frequency of autoimmune diseases is higher, but there is the possibility of misinterpretation of dysplastic bone marrow changes attributed to the advanced patient age or to the underlying autoimmune disease as indicative of primary MDS [17]. In a large French multicenter retrospective analysis of 123 MDS patients, exhibiting systemic inflammatory and autoimmune diseases (SIADs), vasculitic syndromes were more frequently encountered among CMML, and a comparison of this group with 665 patients without such manifestations revealed that patients exhibiting SIADs were younger, male, without RARS, with higher risk disease, and a poor karyotype, but without survival difference [20].

Autoimmune manifestations may not be a single clinical syndrome, but a clustering of two or more autoimmune or immune-based conditions may occur in the same patient. Autoantibodies most frequently found are either organ-specific or non-organ-specific, such as rheumatoid factor, antinuclear antibodies, antineutrophil cytoplasmic (cANCA), or antineutrophil perinuclear antibodies (pANCA), the last two been associated with various vasculitides. Interferon regulatory factor-1 (IRF-1) mRNA expression was found 10-folds increased in MDS patients with autoimmune manifestations, in sharp difference to those without, and to normal controls. It was therefore suggested that absence of IRF-1 expression may be a protective mechanism preventing autoimmunity in MDS [21]. However, from the prognostic point of view although patients with autoimmune manifestations have similar overall survival compared to patients without those with higher IRF-1 expression have longer survival [22].

There is no agreement whether autoimmune manifestations influence prognosis. This is because the severity of autoimmune diseases and conditions, supervened by a MDS, may be substantially diverse and prevent their evaluation as an additional prognostic factor. The majority of retrospective studies tend to demonstrate a survival advantage for patients not exhibiting (auto)immune abnormalities as compared to those who did [23]; however, other studies did not show any difference [9]. In a Japanese study, patients with immune abnormalities had more frequent infections, faster leukemic transformation, and shorter survival. Autoimmune diseases usually respond partially or temporarily to immunosuppressive treatment, but they may relapse and follow the basic disease activity [18]. In other instances they may persist throughout the course of the MDS and demand unaffordable corticosteroid doses to be controlled. In any case, autoimmune diseases remit permanently with allogeneic stem cell transplantation. Remission of autoimmune manifestations has been associated with



improved survival, although in some studies it may accelerate evolution to AML. Furthermore, achievement of remission and return to the MDS stage may induce relapse of the autoimmune condition and in general the manifestation of autoimmunity follows the dysplastic phase and disappear during evolution to AML [24].

In the largest retrospective epidemiologic and prognostic analysis of about 1400 patients, 28% exhibited hyper/autoimmune manifestations and the most prominent was hypothyroidism associated with Hashimoto's thyroiditis (12% of the total population or 44% of the autoimmune syndromes), followed by immune thrombocytopenia (12%), rheumatoid arthritis (10%), and psoriasis (7%). Autoimmune conditions were more frequent among females with lower risk disease, and less transfusion dependent. The probability for AML transformation was lower and the median survival significantly higher for patients with autoimmune diseases (60 versus 45 months), and in multivariate analysis, adjusted for age and IPSS, the manifestation of an autoimmune syndrome was an independent favorable prognostic factor [23].

## 4. Numerical abnormalities of lymphocytes in patients with MDS

### 4.1. T-lymphocyte abnormalities

Many investigational studies have focused on various parameters of adaptive immunity in MDS. In the majority of patients, peripheral blood lymphopenia, mainly CD4<sup>+</sup> cell lymphopenia, and to a lesser degree or at all, CD8<sup>+</sup> cell reduction, frequently resulting in reduction or inversion of the CD4/CD8 cell ratio has been recognized. These findings have not been associated with specific FAB subtypes or any other clinical or laboratory feature [10, 20]. Many studies have confirmed the previous findings, particularly in patients with RAEB, and demonstrated severe functional T-cell impairment in terms of sluggish reaction to mitogenic stimuli and increased radiosensitivity, reflecting impaired DNA repair, implying that these defects might impact on patient hematopoiesis [25].

An initial approach for the interpretation of the numerical imbalance of T-cell subsets was that they might be attributed to multiple red blood cell transfusions, since in some early studies a correlation of the severity of T-lymphocyte abnormalities with transfusion intensity was reported [26]. However, it soon became clear that T-lymphocyte imbalance was present already at baseline, before any medical intervention, and therefore this finding might most probably be a disease feature. T cells of MDS patients synthesize lower amounts of the TH1 direction cytokines interleukin-2 (IL-2) and interferon-gamma (IFN- $\gamma$ ), following mitogenic stimulation, respond inadequately to IL-2 and cooperate inefficiently with B lymphocytes in the induction of immunoglobulin production [24–28]. Studies of NK cell function have always reported reduced cytotoxic and cytolytic activity against cellular targets, as well as impaired both complement-dependent (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) [27]; however, in many other more recent studies, no abnormality in NK cells has been identified. We have investigated several immune function parameters at baseline on the same population of 81 patients with various MDS subtypes and we have shown that patients with RAEB had more profound CD3<sup>+</sup> and CD4<sup>+</sup> lymphopenia, and significantly lower and

sometimes inverted CD4/CD8 cell ratio. We have also shown that CD3+ and CD8+ cell lymphopenia were associated with more frequent infections, higher AML evolution rate, and shorter overall survival [28]. A Japanese group confirmed decreased CD8+ cells in RA patients and inverted bone marrow CD4/CD8 cell ratio, with increased activated CD8+CD11α+ cells in all patients. RAEB patients had decreased marrow total T cells and all MDS patients had decreased marrow CD4+CD45RA+ naïve- and increased CD4+CD45RO+ memory T-helper cells, probably indicating impaired immune surveillance permitting the undisturbed evolution of the dysplastic clone. The prognostic significance of the numerical T-cell abnormalities on the above-mentioned issues was confirmed by other groups [19, 29]. Evaluation of lymphocyte subsets in bone marrow biopsies with specific immunostaining has not demonstrated any quantitative or qualitative T- and NK-cell abnormality, but only revealed increased B lymphocytes in patients with higher risk disease and it has been suggested that identification of >3% B lymphocytes in the marrow biopsy is an adverse prognostic feature [30].

FAB	Reaction		Total Erythema (mm) / Positive Antigens (mean)	Composite score	p
	(+/-)	(+) %			
RA	8/12	66.7	11.9/3.00	18.2 ± 2.7	0.160
RARS	4/10	40.0	8.6/2.40	12.1 ± 1.9	<b>0.003</b>
RAEB	10/16	62.5	11.2/2.43	16.1 ± 2.2	<b>0.029</b>
RAEBt	2 / 8	25.0	6.3/2.25	10.7 ± 1.5	<b>0.002</b>
CMML	5 / 8	62.5	8.8/2.87	14.8 ± 0.7	<b>0.022</b>
Total	29/54	53.7	9.8/2.59	14.8 ± 1.0	0.0003
Controls	16/18	88.9	17.4/4.11	25.8 ± 2.4	

*Notes:* Results of the skin reaction to the Multitest CMI test, consisting of 7 common antigens (Tetanus, Diphtheria, Tuberculin, Candida, Trichophyton, Streptococcus, Proteus): patients with MDS as one group exhibited significantly reduced composite score, compared to healthy controls, matched for age and gender. Significant differences were found for all FAB MDS categories, with the exception of RA (unpublished data). Bold letters indicate statistically significant differences.

**Table 2.** Results of the skin reaction to Multitest CMI® in patients with MDS

Patients with MDS-RA and with aplastic anemia exhibit Th1 and Tc1 polarization of their immune activation [31]. Later this was confirmed also for patients with refractory cytopenia with multilineage dysplasia (RCMD) and was correlated with very high serum and marrow IFN-γ and tumor necrosis factor-α (TNF-α) levels, and high degree of apoptosis. Higher Th1/Th2 and Tc1/Tc2 ratios have been observed in patients with lower risk IPSS and normal karyotype, but not in aneuploid karyotypes. Th1 polarization may not be a uniform finding in MDS patients, but concerns only a subgroup of RCMD with prominent CD8+ lymphopenia [32].

Activated T lymphocytes do not belong to the dysplastic/leukemic clone and express HLA-DR, CD25, CD45RO, and CD57, but not CD28 and CD62L. This antigenic profile is independent of disease subtype, prognostic classification, kind of cytogenetic abnormality, or any other feature [33]. Interestingly, both patients with lower risk MDS and those with aplastic anemia have increased T-lymphocyte counts and B lymphocytopenia compared to patients with high-risk MDS and to controls, but lower risk MDS patients exhibit stronger and uniform Th1/Tc1 polarization than those with aplastic anemia [34]. Moreover, bone marrow NK T-cell infiltrates express the activated effector T-cell phenotype CD8+CD57+CD28–CD62L– and the NK C-lectin-family receptors NKG2D and CD244. These infiltrates represent oligoclonal expansions of autoreactive T cells, as this can be demonstrated by TCR clonality assays and are more prominently identified in the bone marrow than in the peripheral blood [35].

MDS patients also exhibit impairment of delayed cutaneous T-cell hypersensitivity, as this can be demonstrated with various skin patch tests, challenging reaction to common antigens. Most importantly, they may lose immunologic memory against potentially important antigens, such as tuberculin and *clostr. tetani* anatoxin, the clinical consequence of which is unclear [36]. **Table 2** resumes the results from our study of skin reactions to the multitest CMI® in 54 patients with MDS compared to 20 controls (unpublished data).

Further understanding of the immune dysregulation of MDS was achieved through investigation of the regulatory T cells (T-regs). T-regs are a specific subset of helper T cells, inducing immune tolerance and moderating the intensity of immune reactions. Many autoimmune and neoplastic diseases are associated with T-reg impairment, favoring uncontrolled immune activation and attenuation of immune surveillance against tumor growth. An increase of polyclonal/nonclonal T cells in higher risk MDS, and a significant correlation of T-reg number, with the percentage of marrow blasts, the IPSS and progression to AML has been reported [37]. T-regs, characterized as CD4+ CD25<sup>high</sup>+FOXP3+ or CD4+CD25<sup>high</sup>+CD127<sup>low</sup> cells, were found increased in lower risk MDS but they were not correlated with any known disease feature or lab finding [38]. Investigation of the T-reg kinetics, function, and trafficking has revealed that in early MDS, peripheral blood and marrow T-regs are normal in number but dysfunctional, exhibiting lower CXCR4 expression and impaired marrow homing. In contrast, at late MDS or at leukemic transformation, T-regs increase and become functional and migrating. Effective treatment partially restores the number, but disease relapse is again associated with T-reg expansion. Thus, T-regs may share a pathophysiological role in MDS, since impaired suppressor function results in autoimmune phenomena, whereas in more advanced stages, their expansion favors clonal development and leukemic transformation [39]. It has been suggested that absolute number of a T-reg subpopulation, the “effector regulatory T cells,” characterized as CD4+FOXP3+CD25+CD127<sup>low</sup>CD45RA–CD27– cells, could be used as a prognostic factor in lower risk MDS predicting severity of anemia, AML transformation, and overall survival [40].

Other interesting T-cell subsets are the IL-17 producing helper T cells, the so-called Th17 cells and the Th22 cells. Th17 cells were found substantially increased in patients with lower risk MDS and their number was inversely correlated with that of T-regs. T-regs, although suppressive for other T-cell populations, do not affect Th17 cell number. Thus, the Th17/T-reg cell ratio has been found very high in lower risk disease and has been proposed as a marker of

“effective” immunosuppression, high degree of apoptosis, and higher risk for autoimmunity, as well as an indicator for application of immunosuppressive treatment [41]. In contrast, helper T cells producing IL-22 (Th22 cells), involved in the pathogenesis of inflammatory reaction and autoimmunity, were found increased in patients with advanced MDS and their number was correlated with the mRNA levels of proinflammatory cytokines [42].

Finally, peripheral blood  $T_{\gamma\delta}$  lymphocytes, possessing a TCR with rearranged gamma/delta chains, and particularly  $V_{\gamma 9}V_{\delta 2}$  T cells, the major  $T_{\gamma\delta}$ -cell subset, which represent an important subpopulation for antitumor activity, were found reduced in patients with MDS and the reduction was greater in patients exhibiting autoimmune manifestations. Although  $T_{\gamma\delta}$  cells were not clonal, they reacted poorly to IL-2, and bromohalohydrin, a specific mitogen for these cells, induced mitogenic responses in only 60% of the MDS studied, unrelated to any specific disease feature. However, when activated, they exerted normal antileukemic effects against leukemic blasts. Therefore, the impaired number and function of this T-cell subpopulation may play a role in clonal expansion and disease progression of MDS [43].

#### 4.2. B-lymphocyte abnormalities

Although B lymphocytes in MDS patients do not demonstrate the spectrum of abnormalities detected in T cells, since their production and function is governed by T cells, their aberration actually reflects the functional integrity of T cells. Information for B lymphocytes is fewer and often conflicted. In many studies, decreased proportion and peripheral blood absolute B lymphocytopenia, frequently accompanied by hypogammaglobulinemia and recurrent infections has been reported [27, 44]. These findings are mostly confined to patients with lower risk disease and are associated with T-lymphocyte imbalance and reduced numbers of bone marrow B cells and B-cell precursors.

Analysis of the marrow CD34+ cell differentiation toward B lymphocytes in patients with low-risk MDS has revealed low expression of B-lineage differentiation genes and reduced production of B-cell precursors, a finding proposed to be used as a hallmark of low-risk disease [45]. An additional factor contributing to B lymphocytopenia is that bone marrow B-, but not T lymphocytes, exhibit increased apoptosis, similar to that observed in nonlymphoid cells. Increased B-cell apoptosis could not be considered a clonal “property,” since it was found neither in leukemic nor in normal marrows [44]. This was also confirmed on trephine biopsies of patients with high-risk MDS only, and the percentage of B lymphocytes was inversely correlated with prognosis [30]. Bone marrow flow cytometry analysis has shown increased proportion of CD34+CD45<sup>low</sup> B-cell precursors in patients with RA and RARS, and lower values in those with RAEB, whereas in AML B-cell precursors were not found at all, probably reflecting differentiation incapability of the CD34+ cells. Indeed, an inverse correlation of CD34+CD45<sup>low</sup>+ B cells with marrow blasts and a positive one with hemoglobin was found. Abnormal expression pattern of B-cell differentiation antigens, with hypoexpression of CD79 $\alpha$  and TdT has also been reported, implying a possible role of the MDS marrow micro-environment in the maturation process of B lymphocytes [46].

Regarding the origin of B- and T lymphocytes, available data are again conflicting. In the majority of clonal studies, neither T- nor B lymphocytes or NK cells were found to originate



from the dysplastic clone. Some studies have shown clonal origin of the B lymphocytes in a proportion of MDS patients, and in a Japanese study, the majority of patients with RA and those with immunological abnormalities exhibited clonal B lymphocytes [47]. Clonal origin was also found in 5% of the CD20+/CD22+ B cells of patients with trisomy 8. By using interphase FISH on sorted marrow cells, 13% of the CD5+CD19+ lymphocytes were clonal, implying that a part of the B lymphocytes in some patients may be clonal and that these cells may contribute to the manifestation of immune abnormalities [48].

B lymphocytes of MDS patients express low number of HLA-DR molecules (HLA class-II antigens) and are either deficient of EBV receptors or they carry abnormal Fc $\gamma$  and C3d receptors, which cannot be used by EBV viral particles to enter and activate B cells. B-lymphocyte cultures produce increased amounts of IL-6 and IL-10, following mitogenic stimulation. IL-6 is overproduced even without mitogenic stimuli. Finally, B lymphocytes of patients manifesting immune abnormalities have lower number of cell surface Fas ligand receptors, a finding possibly indicating their resistance to apoptosis.

Not surprisingly several quantitative serum  $\gamma$ -globulin and immunoglobulin abnormalities have been described in MDS patients, such as polyclonal hyper- $\gamma$ -globulinemia, monoclonal M-spikes, or hypo- $\gamma$ -globulinemia. Monoclonal components have been found significantly higher than in normal age-matched population [49]. It has been suggested that dysplastic monocytes might exert unspecific immune stimulation on B- and T lymphocytes through increased IL-1 production favoring the development of monoclonal B-cell populations and producing M-spikes.

Investigating the significance of serum protein electrophoresis in 158 patients, we noticed a normal pattern in 36% (mainly in RA and RARS) and only in 8% of CMML. A normal baseline pattern was associated with longer survival, independently of the IPSS and FAB classification. An acute phase reaction (alpha2-globulins >10 g/l) was seen in 17% at baseline, developed in additional 24% in the course of the disease, but in 80% of the patients transformed to AML and was associated with shorter survival. Hypo- $\gamma$ -globulinemia was found in 6%, mainly RARS, was not related to frequent infections, and in RAEB it was associated with decreased marrow cellularity, deeper cytopenias, and longer survival. Polyclonal hyper- $\gamma$ -globulinemia was found in 41% of patients (particularly RAEB-t, CMML) and monoclonal proteins in 16 cases (10%) more commonly in CMML and 2.5 times more frequently than in a control population of similar age. An additional 18% of the patients exhibited discrete M-components among polyclonal spectrum of  $\gamma$ -globulins. This finding has not yet been described and its significance is unclear [50].

#### 4.3. NK cell abnormalities

Patients with MDS exhibit severe functional NK-cell impairment, but sometimes also numerical abnormalities. Cytotoxic NK-T cells, phenotypically characterized as CD3+CD8+CD16+, are usually normal or decreased but IFN- $\gamma$  production is normal or increased. NK cells, characterized as CD3-CD8-CD11b+HNK1+ CD56+CD57+ cells, have been found normal, rarely decreased, but sometimes even increased [51]. The NK activity of MDS patients is almost always decreased compared to healthy controls [27, 51], although immunophenotypically NK



cells are indistinguishable. In general, CD8<sup>+</sup> T-cell function and the defective NK activity in MDS have been strongly and inversely correlated with bone marrow blast cell percentage, marrow cellularity, and serum sIL-2R levels [51]. Alloantigen- or mitogen-induced cell-mediated cytotoxicity [27] as well as IFN- $\alpha$  and IL-2 production following NK cell activation is also impaired and the preincubation of NK cells with IFN- $\alpha$  may partially increase NK activity [52]. There are conflicting data regarding the origin of NK cells. By using FISH on FACS-sorted cells of patients with monosomy 7, monosomic signs in CD3<sup>+</sup>CD56<sup>+</sup> cells were detected in 3 out of 4 [53]. In another study, between 20 and 50% but not all the NK cells were clonal, demonstrating a kind of “chimerism” by clonal and nonclonal NK cells in the majority of patients.

Many groups investigated whether IFN- $\alpha$  treatment could induce blast/clonal cell clearance, through augmentation of the NK activity. In one study on 38 patients with RAEB, following 3-month treatment with IFN- $\alpha$ , NK activity and NK cell number and function was increased, but these alterations were not associated with any meaningful clinical response. NK cells exhibited normal tumor cell binding capacity, but inability of releasing cytotoxic factors, possibly suggesting intrinsic functional defects [52]. Another group did not confirm any quantitative defect and found normal expression of the activating receptors NKp46, NKp30, and NKG2D, but a depressed cytolytic activity. Incubation with IL-2 upregulated the NKp46 expression, but did not enhance NK-cell cytotoxicity but induced higher rate of apoptosis [53]. A strong correlation of the NK activity with higher IPSS, abnormal karyotype, excess of blasts and marrow hypercellularity, and downregulation of the NKG2D receptor has also been reported [54]. The Nordic MDS study Group showed that decreased expression of DNAM1 and NKG2D receptors on marrow NK cells was inversely correlated with blast percentage and suggested that DNAM1 plays a pivotal role in NK-mediated cell killing [55].

IL-12, alone or combined with IL-2, induces variable and unpredictable response to NK cells. Some patients (mainly with RA) exhibit a response closer to normal, while others respond poorly. The combination of IL-2 and IL-12 increases IFN- $\gamma$  and TNF- $\alpha$  production in a synergistic way. IL-12 alone is not so stimulatory, and the combination of IL-2+IL-12 generates stimulation, similar to that obtained by IL-2 alone. Indeed, priming of peripheral blood mononuclear cells (PBMC) with IL-12 increased their cytotoxicity against autologous leukemic blasts to almost normal levels and significantly reduced WT1 mRNA expression, used as a marker of residual leukemic burden, except in patients with overt, high-bulk AML. Thus, *ex vivo* priming of cytotoxic NK-T and NK cells could be used as a tool, targeting residual disease, following systemic chemotherapy [56].

In a study from Düsseldorf, the authors recognized a small subgroup of high-risk patients, with almost absent peripheral blood NK cells, but intact populations of NK T cells. A larger subgroup with normal number but poor function of NK cells was characterized by reduced intracellular granzyme-B and perforin levels. This subgroup restored almost completely NK-cell function, following mitogen or cytokine stimulation. NK cells were mainly immature but exhibited normal mature/activated (CD56<sup>bright</sup>+CD107<sup>+</sup>) immunophenotype and a restricted repertoire of KIR receptors. It is therefore suggested that the dysfunctional NK cells lead to inefficient/insufficient immune surveillance and clonal expansion [57]. The Pittsburgh Group

reported different marrow frequencies of NK and NK T cells in MDS and AML. In MDS they did not find numerical impairment of the NK-cell population, but a significant decrease in mature CD56<sup>dim</sup>CD16+CD57<sup>bright</sup> cells, which had great prognostic significance for survival [58]. Other groups have reported increased intracellular granzyme-B levels in the NK cells of MDS patients [59].

## 5. Serum cytokine profiles

The immune-activated status of MDS patients lead to overproduction and elevated serum levels of many cytokines. We were the first group to report elevated serum soluble interleukin-2 receptors (sIL-2R) and tumor necrosis factor- $\alpha$  levels in 42 MDS patients confirming an abnormal immune stimulatory status. Although the difference in TNF- $\alpha$  levels between early and advanced MDS was not significant, patients with advanced MDS had significantly higher serum sIL-2R levels compared to those with early MDS [60]. *In vivo* treatment with rhGM-CSF or high-dose IL-3 further increases sIL-2R levels, which are associated with higher marrow cellularity and blast cell percentage, faster AML evolution, and shorter survival. These findings possibly reflect quantitative and qualitative abnormalities of the CD8+ and NK-cell subsets, resulting in ineffective T-B cell communication and impaired NK-cell function, since sIL-2R antagonizes the cellular receptor in IL-2 uptake, restricting T-cell activation [61]. sIL-2R levels are negatively correlated with T- and NK-cell counts and positively with adverse events occurring in the course of lower risk patients for whom sIL-2R levels are an independent adverse prognostic factor.

Serum IL-6 levels were found elevated in the majority of MDS patients and serum GM-CSF levels in less than half of them, although these cytokines were undetectable in normal subjects. Higher IL-6 concentrations were found in patients with advanced subtypes, were inversely correlated with the severity of the anemia and positively with peripheral blood and bone marrow blast cell percentages, and may increase further following chemotherapy. IL-6, IL-7, MCSF, TGF $\beta$ , and IL-1 $\beta$  are constitutively produced by marrow stromal cells of patients with MDS and AML, but not from stromal cells of normal subjects, and IL-6 gene transcription could be induced by exogenous addition of IL-1 $\beta$  confirming a cytokine network dysregulation [62]. Serum IL-8 levels were also found elevated, but they dropped under chemotherapy or during remission.

A Dutch group measured serum levels of seven cytokines in 75 MDS patients and found detectable levels of G-CSF in the majority of them, and increased IL-3 and IL-6 levels in a minority of patients but not in controls [63]. Serum TNF- $\alpha$  levels have been correlated with the severity of anemia, poor performance status, leucocytosis and monocytosis, higher  $\beta$ 2-microglobulin and lower albumin levels, liver and renal impairment, and shorter survival [62–64]. TNF- $\alpha$  levels <10 pg/ml have been associated with achievement of higher remission rate and longer PFS Progression Free and overall survival, whereas lower TNF- $\alpha$  and IL-1 $\beta$  levels could predict response to treatment with erythropoietin [64]. Thus, TNF- $\alpha$  represents the most important circulating and measurable cytokine, from the pathogenetic and the prognostic

point of view. In general, serum levels of type-1 cytokines (IL-1 $\beta$ , IL-7, IL-8, IL-12, RANTES, and IFN- $\gamma$ ) [64, 65] are found elevated in lower risk MDS, whereas inhibitory factors (IL-10, sIL-2R) are elevated in higher risk disease.

The group of Mayo Clinic evaluated plasma levels of 30 different cytokines in 78 patients, and showed that although levels of 19 cytokines differed significantly from controls, in multivariate analysis, only levels of IL-6, IL-7, and CXCL10 had independent prognostic value for survival. Indeed, patients with normal levels of all these three cytokines had a median survival of 76 months compared to only 25 months for patients with elevated levels of at least one of them. For IL-6 levels in particular, a strong association with inferior leukemia-free survival, independent from other prognostic factors, was found [66].

Finally, a Spanish group, among other findings, demonstrated an inverse correlation of the CD3+, CD4+, and CD8+ populations with age, as well as an inverse correlation of serum IL-10 levels with the number of CD8+ cells, disease progression, and overall survival [67]. In another study, investigating the association of IL-10 gene polymorphisms with the development and the features of MDS, the highly IL-10-expressing genotype -592 CC was associated with more severe anemia and poorer survival compared to non-IL10-expressing genotypes, thus confirming a significant prognostic role for IL-10 [68].

## 6. Functional immunoregulatory abnormalities of T lymphocytes

### 6.1. Mixed lymphocyte reactions (MLRs): basic information

A basic property of the immunocompetent cells is the recognition of the “self” and the orchestration of an immune response against the “nonself” or the “altered self,” and when self-recognition is impaired, an autoimmune disorder emerges. An initial interpretation for the frequent autoimmune disorders and other immune abnormalities of MDS patients was that they might probably reflect clonal origin of B- and T lymphocytes. Later, however, it was demonstrated that, in the majority of cases, B- and T lymphocytes are nonclonal, but T-lymphocyte abnormalities may influence disease course. Various T-lymphocyte subsets exert complex immunoregulatory activities on other T-cell populations, B lymphocytes, and monocytes. Mixed lymphocyte cultures are performed with the coculture of a pure T-cell population (responder cells), upon which a kinetically inactive, but cell-surface intact, non-T cell population (stimulant cells) affects, thus generating a mixed lymphocyte reaction. MLRs represent dynamic *in vitro* models for the study of various cellular interactions and of the immunoregulatory mechanisms developed between different immunocompetent cell populations. When the stimulant and the responder cell population stem from the same subject, the model is called *autologous MLR* (AMLR), whereas when the stimulant population stems from another subject the model is called *allogeneic MLR* (Allo-MLR). AMLR and Allo-MLR constitute practical tools for the investigation of various diseases and conditions with an underlying immune-based pathogenesis or pathophysiology.

The proliferative reaction (MLR) is mediated through recognition of structural antigenic domains of the cell surface of non-T cells, and particularly HLA class-II antigens. The stimu-

lating capacity of the non-T-cell population is abrogated when cell membrane structure is destroyed, either following mechanical stress or treatment with proteolytic enzymes. Moreover, the stimulatory capacity is not a soluble factor and non-T- or B-lymphocyte supernatants do not retain any stimulatory activity on T lymphocytes [69], whereas preincubation of the non-T-cell population with anti-HLA-DR monoclonal antibodies completely abrogates the AMLR and substantially depresses the Allo-MLR. The stimulatory potential of other membrane determinants on the MLRs was identified in a similar way. Such molecules are HLA class I (HLA-A, -B, and -C) for the Allo-MLR, CD3-Ti complex for any type of MLR, and probably additional minor antigenic determinants of the MHC. Stimulating capacity of the non-T-cell population is dependent on the various mononuclear cell constituents included. B lymphocytes are stronger stimulants than NK cells and null lymphocytes. Activated B lymphocytes, surface IgM(+) B lymphocytes, and B lymphoblasts are better stimulants than resting- and IgM(-) B lymphocytes, and this property is independent of their content in EBV-DNA or the origin from a mitogen-enriched culture. The role of monocytes is contradictory, since inactive monocytes enhance autologous reactivity, whereas the admixture of monocytes in the responder T-cell population results in severe impairment of both AMLR and Allo-MLR.

MLRs share the characteristic features of an orchestrated immune reaction showing immunologic memory and specificity. When T lymphocytes, previously exposed to autologous non-T cells and obtained the seventh day of culture, are re-exposed to the same non-T-cell population, they demonstrate their peak proliferation earlier, on the third day of culture (secondary AMLR), thanks to previously engrafted immunologic memory. The same has been confirmed for the Allo-MLR, because when in the secondary culture the allogenic stimuli are different, then different responder T-cell population is activated and the reaction shows the kinetic of the primary MLR. There are different autoreactive and alloreactive T-cell populations. The number of alloreactive T cells is 5–40 times higher than autoreactive, and represents 1/400–1/150 of the total peripheral blood T lymphocytes, whereas autoreactive constitute 1/5000–1/2200 of them.

The basic function of the MLRs is the production of suppressor “activity” or of suppressor/cytotoxic T cells. The main part of the responder population are CD4+ helper/inducer T lymphocytes and treatment of this population with an anti-CD4 monoclonal antibody, practically abrogates all types of MLR. Conversely, treatment with an anti-CD8 antibody quantitatively decreases the strength of both types of MLR. Thus, from the relative content of the two major T-cell subpopulations AMLR has two different phases. Responder CD4+ T lymphocytes undergo a proliferative reaction upon sensation of autologous signals (self-MHC antigens: autoreactive T cells). CD4+ cell proliferative reaction is peaked on the third and fourth day of the culture, when helper T-cell population dominates. This reaction is followed by a secondary activation of the suppressor CD8+ T cells, which is quantitatively stronger, is peaked on the seventh and eighth day of the culture and inhibits any further proliferation of the autoreactive T cells. This serially fulfilled lymphocyte reaction is mediated through the production of IL-2 by the CD4+ T cells. In the Allo-MLR the responder population (alloreactive T cells) is activated through the recognition of the MHC alloantigens and is consisted of both helper and suppressor T lymphocytes. Allo-MLR is always stronger than AMLR. Deficiency



of AMLR and of Allo-MLR has been reported in various diseases and conditions, a list of which is provided in **Table 3**.

<b>Connective tissue disorders</b>	<b>Autoimmune diseases</b>
Rheumatoid arthritis	Immuno-hemolytic anemia
Still's disease	Immune thrombocytopenic purpura
Systemic Lupus Erythematosus	Henoch-Schoenlein purpura
Dermatomyositis/polymyositis	Insulin-dependent diabetes mellitus
Sjogren's syndrome	Hashimoto's thyroiditis
Ankylosing spondylitis	Chronic active hepatitis
	Inflammatory bowel disease
<b>Infectious diseases</b>	Primary biliary cirrhosis
Infectious mononucleosis	Myasthenia gravis
Chronic mucocutaneous candidiasis	Multiple sclerosis
Acquired immunodeficiency syndrome	Arthropathic psoriasis
Chronic Hepatitis C	
Chronic periodontitis	<b>Allergic diseases and conditions</b>
	Allergic asthma
<b>Neoplastic diseases</b>	Atopic dermatitis
Breast cancer	Hay fever
Lung cancer	Food allergy
Colon cancer	
Head and neck cancer	<b>Various diseases and conditions</b>
Gastric cancer	Sarcoidosis
Bladder cancer with schistosomiasis	Down's syndrome
Kaposi's sarcoma	Congenital immunodeficiency
Myelodysplastic syndromes	Idiopathic portal hypertension
	Ataxia-telangiectasia
<b>Lymphoproliferative disorders</b>	Congenital hyper-IgM syndrome
Chronic lymphocytic leukemia	Mixed cryoglobulinemia
Hairy cell leukemia	Hemophilic patients treated with fVIII concentrates
Hodgkin's lymphoma	Transplanted patients
B-cell non-Hodgkin's lymphoma	Patients in chronic hemodialysis
Peripheral T-cell lymphoma	Advanced age
Multiple myeloma	

**Table 3.** Diseases with an abnormal autologous mixed lymphocyte reaction.

Besides the immunoregulatory cell circuits, generated following “autorecognition” in the AMLR, helper and suppressor T lymphocytes exert regulatory function in normal hematopoiesis. T lymphocytes obtained at the early phase of the AMLR (third day) have promoting



activity on the formation of early (bursts) and late erythroid colonies (CFU-E) and this activity is similar to that obtained by PHA-activated T lymphocytes. This activity, initially termed *Burst Promoting Activity*, is attributed to the production of various hematopoietic cytokines and particularly interleukin-3. Following the development of suppressor activity for dampening autologous reaction on the seventh culture day, this activity also induces suppression of development of immature erythropoietic and other progenitor cells, similar to that generated from activated lymphocytes following prolonged antigenic stimulation, as this happens in chronic infections, inflammatory conditions, connective tissue diseases, and in aplastic anemia. In these situations activated suppressor T lymphocytes produce suppressive cytokines, and particularly but not exclusively IFN- $\gamma$ .

Cytotoxic activity, generated in the MLRs, is also directed against autologous and allogeneic B lymphocytes, monocytes, and cells with an altered antigenic profile, either neoplastic or not. Generation of cytotoxic activity against tumor cells as a result of immune activation is of tremendous clinical significance. In AMLR and Allo-MLR the neoplastic cells may represent the stimulant cell population, whereas responder populations might be both the suppressor/cytotoxic CD3+CD16+ NK-T cells and the CD3-CD16+CD56+ NK cells. Activation of these populations results in increased proliferation and the adoption of an activated profile. Thus, cytotoxic T cells generated in AMLR may play an important role in antitumor surveillance [70].

## 6.2. Autologous and allogeneic MLRs of patients with myelodysplastic syndromes

AMLR and Allo-MLR were found significantly reduced on 12 MDS patients and the amount of IL-2 produced during these reactions was severely depressed. Exogenous addition of IL-2 partially restored the strength of the reactions, which, however, continued to be substantially reduced compared to normal controls. To investigate which cell population was primarily affected, the authors compared the strength of Allo-MLR by using allogeneic non-T cells from both normal controls and other MDS patients against T cells from MDS patients. They also tested Allo-MLR of T lymphocytes from healthy controls against non-T cells obtained either from normal subjects or from MDS patients. Allo-MLR of T cells from MDS patients was substantially improved with the use of normal allogeneic non-T cells, but did not reach the normal range. Conversely, Allo-MLR of T cells from normal subjects was severely deficient when stimulant non-T cells had been obtained from MDS patients compared to the reaction against non-T cells from normal subjects. Therefore, it appears that in MDS there is an impaired stimulating capacity of the non-T-cell population consisting of B lymphocytes, monocytes, and immature myeloid cells [26]. Almost concurrently the presence of "leukemia-inhibitory activity" (LIA) of peripheral blood non-T cells of MDS patients mainly obvious in patients with an excess of blasts, but also in some patients without an excess of blasts (RA-RARS) was reported. Moreover, the majority of the patients without an excess of blasts, whose serum contained LIA, evolved quickly to RAEB/AML. This "activity" of higher risk MDS patients could be eluted from culture of PBMC in FCS-enriched media with the addition of GM-CSF and IL-4 [71]. Cells responsible for the induction of suppression/inhibition of cell growth are clonal macrophages, transformed in culture to "giant macrophages" or dendritic cells. The

mediator of suppression was a soluble factor, other than IFN- $\gamma$  or TNF- $\alpha$ , identified as acidic isoferitin [72].

We investigated the MLRs in 20 MDS patients in paired experiments with sex- and age-matched controls at baseline, before the administration of any interventional treatment. To express the strength of reactions we used the *Stimulation Index*, i.e., the ratio of the incorporated  $^3\text{H}$ -thymidine in the MLR divided by the incorporated  $^3\text{H}$ -thymidine in an unstimulated culture of equal number of purified CD3+ T lymphocytes. Patients with MDS exhibited severely impaired AMLR in all experiments with a median value almost half as that of the controls, without overlapping values, and the difference between the two groups was statistically very significant ( $p < 0.000001$ ). Patients with RAEB showed that the most attenuated reactions were significantly weaker than the remaining patients [73]. Cumulative results are shown in **Table 4**.

FAB group	N	(Mean $\pm$ SEM) cpm	(Mean $\pm$ SEM) S.I.	p	AMLR patient/AMLR control
RA	4	2068 $\pm$ 205	2.64 $\pm$ 0.33	<b>0.0023</b>	0.58 $\pm$ 0.09
RAS	5	2301 $\pm$ 206	2.77 $\pm$ 0.18	<b>0.0012</b>	0.63 $\pm$ 0.06
RAEB	8	1837 $\pm$ 310	1.90 $\pm$ 0.07	<b>&lt;0.0001</b>	0.40 $\pm$ 0.03
CMML	3	2562 $\pm$ 177	3.39 $\pm$ 0.21	<b>0.0413</b>	0.51 $\pm$ 0.08
<b>All patients</b>	20	2108 $\pm$ 154	2.49 $\pm$ 0.15	<b>&lt;0.0001</b>	0.51 $\pm$ 0.04
<b>Controls</b>	20	4396 $\pm$ 404	5.31 $\pm$ 0.32		
<b>RAEB vs. other MDS pts (S.I.): 1.90 <math>\pm</math> 0.07 vs. 2.88 <math>\pm</math> 0.16</b>				<b>0.0004</b>	

**Table 4.** Results of the autologous mixed lymphocyte reaction in patients with MDS (counts per min and stimulation index, S.I.) Bold letters/numbers indicate statistically significant differences.

To evaluate the capability of the stimulant cell population, Allo-MLR was performed against non-T cells originating either from another MDS patient or from a healthy control. Moreover, to evaluate the capability of the responder cells, Allo-MLR of the healthy controls was performed against non-T cells from MDS patients or from other controls. In all cases, Allo-MLR against normal non-T cells was substantially higher, and on average threefold as strong as AMLR and Allo-MLR against “dysplastic” non-T cells was weaker, but always stronger than AMLR of the same person. The difference between these two types of controls’ Allo-MLR was significant (S.I.: 7.90  $\pm$  0.89 versus 14.12  $\pm$  1.59,  $p = 0.0035$ , unpublished data). When compared to controls, Allo-MLR of MDS patients was significantly impaired in all comparisons (S.I.: 4.53  $\pm$  0.41 versus 14.12  $\pm$  1.59,  $p = 0.000014$ , unpublished data). Significant difference was maintained in the comparison of Allo-MLR between healthy controls and patients with RA, RARS, and RAEB separately, whereas CMML patients exhibited the less, and RAEB patients the most attenuated reactions, significantly weaker than the remaining MDS. In paired analysis, alloreactivity of MDS patients was always weaker than that of the corresponding control and the ratio Patient’s Allo-MLR/Control’s Allo-MLR was always  $<1$  (median 0.36, range 0.06–0.58). Among MDS patients, “dysplastic” origin of the non-T cells did not further

impair the already depressed alloreactivity. However, even in this Allo-MLR the difference between patients and controls was still significant (S.I.:  $3.64 \pm 0.21$  versus  $7.90 \pm 0.89$ ,  $p = 0.026$ ) [73]. Results of the Allo-MLR are shown in **Table 5**.

FAB Group	Normal non-T cells				Dysplastic non-T cells			
	N	$\times \pm \text{SEM}$	p	S.I.	N	$\times \pm \text{SEM}$	p	S.I.
		cpm				cpm		
RA	4	$3562 \pm 334$	<b>0.020</b>	$4.79 \pm 0.64$	3	$2532 \pm 407$	n.s.	$3.01 \pm 0.23$
RAS	5	$4569 \pm 702$	<b>0.021</b>	$5.86 \pm 0.87$	4	$3859 \pm 685$	n.s.	$4.89 \pm 1.16$
RAEB	8	$2643 \pm 328$	<b>&lt; 0.000</b>	$3.04 \pm 0.25$	7	$1865 \pm 563$	n.s.	$2.21 \pm 0.49$
CMMML	3	$4215 \pm 695$	0.070	$5.93 \pm 0.62$	1	3380	–	4.47
All Pts	20	$3544 \pm 312$	<b>&lt; 0.0001</b>	$4.53 \pm 0.41$	15	$2697 \pm 389$	<b>0.040</b>	$3.63 \pm 0.21$
Controls	20	$11,355 \pm 1459$		$14.12 \pm 1.59$	12	$6558 \pm 869$		$7.90 \pm 0.89$
RAEB vs. all other MDS (S.I.)			<b>&lt;0.001</b>	$3.04 \pm 0.29$				$5.52 \pm 0.47$

**Table 5.** Allogeneic mixed lymphocyte reaction in patients with myelodysplastic syndromes – counts per min and stimulation index (S.I.) Bold letters/numbers indicate statistically significant differences.

Similar results were obtained by the Czech group who found significantly decreased MLRs with lower TNF- $\alpha$  and IFN- $\gamma$  production in the supernatants of patients with RA compared to the MLRs of RARS patients. They also found less affected the allo-MLR against normal non-T cells, and identified as more defective the second (effector) phase of the reaction [74]. Therefore, in the MLRs of MDS patients there is an impairment of both the responder (T cells) and the stimulant population (non-T cells). The responder population reacts poorly to autologous and allogeneic stimuli and exhibits a profile of immune tolerance, which is clearer in the high-risk patients. Moreover, the stimulant population provides insufficient stimuli for reaction to the T cells, since it also depresses the alloreactivity of normal T lymphocytes. The possible, if any, clinical consequences of these findings are practically unknown or remain only speculative.

**6.3. Pathogenesis of immune dysregulation in MDS: immune abnormalities or immune adaptation?**

*6.3.1. Autoreactivity against the clone: Autologous progenitor cell/T-lymphocyte reaction (APLR)*

“Inhibitory activity” derived from serum and PBMC culture’s supernatants of MDS patients has earlier been described and associated with poor prognosis [71]. Normal PBMC inhibit autologous hematopoietic cell colony formation in short-term cultures, as did also PBMC of patients with RA, but they induced a clear inhibitory activity later on day 10. Responsible cells are probably cytotoxic T and NK cells, which may react against the clone and suppress the growth of clonal cells at early stages of the disease. If this suppressor function develops early and is effective, clonal growth may be arrested. Nevertheless, NK cells of MDS patients usually

exhibit impaired function and sometimes are clonal. However, since suppressor activity is achieved through various soluble cytokines and mainly through TNF- $\alpha$ , it is not quite specific and may also affect nonclonal cells, resulting in hematopoietic suppression, as this is observed in hypoplastic MDS and aplastic anemia. Indeed, lymphocyte culture supernatants from MDS patients exert suppressive activity on the growth of normal hematopoietic progenitors [75].

This form of cytotoxicity was also identified in high-risk MDS and in AML, and was attributed to possible infection of leukemic cells by an oncogenic virus. However, viral infection is not necessary for the generation of an immune reaction, since clonal cells contain and sometimes express on their membrane many abnormal or mutated proteins, possibly representing neoantigens capable to induce lymphocytotoxic reactions by CD8+ T cells. Autoantigens are hardly found in MDS and may only be speculative but have been identified in some other marrow failure syndromes, such as aplastic anemia and paroxysmal nocturnal hemoglobinuria. As possible antigens, the Wilms Tumor protein (WT1), moesin, a cytoskeleton protein, KIF20B (kinesin), desmoplakin, and proteinase-3, an enzyme of blast-cell granules, have been indicated [76]. T lymphocytes of some MDS/AML patients stimulated *in vitro* with WT1 and proteinase-3 were polarized toward TH1 direction with the production of IFN- $\gamma$  and the enrichment of their cytoplasm with granzyme B [77]. Moreover, patients expressing defined proteinase-3 aptotype generate stronger allogeneic lymphocytotoxic reactions following allogeneic hematopoietic stem-cell transplantation (GVL effect).

A challenging hypothesis is that the adaptive immunity may rather “react” than be impaired following various cellular interactions, and this immune “reaction,” or at least such cellular interactions, might be a part of the pathogenesis of MDS. This “reaction” also may represent a defensive mechanism of the immune system against the dysplastic/neoplastic clone and is orchestrated specifically against clonal bone marrow cells. Specific CD8+ suppressor/cytotoxic T cells recognizing progenitor cells with trisomy 8 have been identified in MDS patients with this abnormality. Clonal inhibition is achieved via MHC class I recognition and through induction of FAS-mediated apoptosis [78]. The possible contributing role of an altered marrow microenvironment in the development of such immune alterations is also tempting.

The presence of increased number of immunocompetent T lymphocytes with an activated cytotoxic immunophenotype CD8+CD25+CD28-CD57+ has been reported in the marrows of patients with aplastic anemia and MDS. These cells do not directly influence the severity of peripheral blood cytopenias [79]. In our study on 41 patients, the percentage of activated marrow suppressor/cytotoxic T lymphocytes was inversely correlated with marrow cellularity and blast cell percentage, and positively with Fas antigen expression on CD34+ clonal progenitor cells [80]. The cytotoxic reaction against marrow CD34+ cells of MDS patients has a well-defined signal transduction pathway in the T cells and can be augmented *in vitro* with the exogenous addition of IL-2 [81]. The strength of this reaction has not been associated with any TNF- $\alpha$ -, IL-10-, or lymphotoxin gene polymorphism, although as it is well-known that these polymorphisms appear to influence the severity of acute GVHD, following allogeneic hematopoietic stem-cell transplantation [82].

We also investigated the behavior of the clonal CD34+ progenitors as stimulant population in mixed cultures with autologous T lymphocytes as responder cells, in other words the immune



reaction when T cells are in close contact with clonal stem cells. We compared this type of reaction (autologous progenitor cell mixed lymphocyte reaction—APLR) with the classical types of MLRs. APLR reflects the strength of the immune reaction against the clone in a background of established relative immune tolerance. We tested APLR in 20 MDS patients and 10 healthy controls. We noticed significant differences in the strength of the reaction between patients and controls, as well as between the various subtypes of MDS. Results are shown in **Table 6**. Among normal subjects APLR was rather a mild proliferative reaction, less than half strong as AMLR and about six- to sevenfold weaker than Allo-MLR. Among MDS patients, APLR was significantly stronger than in controls ( $p = 0.048$ , unpublished data, see **Table 6**). Stimulation index ranged between 1.8 and 26.0 in patients, and between 1.4 and 3.0 in controls. Thus, APLR was the only MLR in which MDS patients exhibited stronger reactions than controls and with high variability [83]. In particular, patients without excess of blasts had APLR similar to normal subjects, whereas patients with RAEB showed significantly stronger reactions. Specifically, a subgroup of four RAEB patients exhibited very strong reactions with a SI >10, significantly higher than the remaining MDS patients, although the same patients developed weak responses against autologous and allogeneic non-T cell stimuli. As mentioned earlier, in all healthy controls the ratio APLR/AMPR was always <1. In patients with RA or RARS this ratio was around 1 but in some of them higher than 1, whereas in patients with RAEB, APLR/AMLR ratio was substantially higher than 1. Thus, the three subject groups tested with MLRs (low-risk MDS, high-risk MDS, and controls) could be compartmentalized in three different areas in the plot (see **Figure 1**).

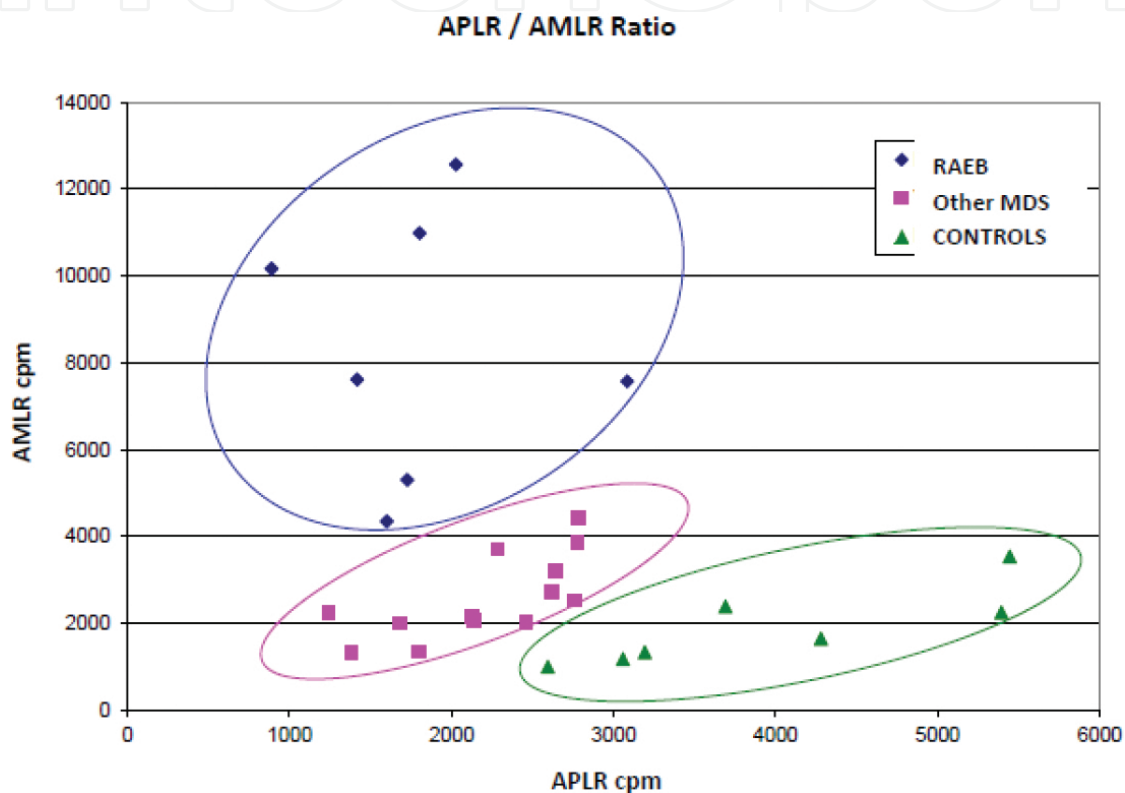
FAB Group	N	cpm (Mean ± SEM)	Stim. Index (Mean ± SEM)	p	APLR pt/APLR control
RA	4	2334 ± 382	2.88 ± 0.41	0.173	1.21 ± 0.11
RAS	5	2365 ± 385	2.77 ± 0.18	0.109	1.34 ± 0.13
RAEB	8	7502 ± 1194	10.35 ± 2.46	<b>0.001</b>	3.92 ± 0.74
CMMML	3	3488 ± 556	4.55 ± 0.25	<b>&lt;0.001</b>	1.43 ± 0.12
All patients	20	4582 ± 735	6.09 ± 1.27	<b>0.048</b>	2.39 ± 0.85
Controls	10	1866 ± 308	2.21 ± 0.31		
RAEB	8	7502 ± 1194	10.35 ± 2.46		
Other MDS	12		3.26 ± 0.95	<b>0.004</b>	

**Table 6.** Autologous progenitor cell mixed lymphocyte reaction (APLR) Counts per min and stimulation index Bold letters/numbers indicate statistically significant differences.

Our results have been confirmed by Chamuleau et al., who demonstrated increased non-MHC-restricted autologous cytotoxicity against clonal marrow precursors in eight patients with lower risk MDS, possibly indicating immune surveillance against clonal expansion although they have not provided clinical data on its significance [59]. Suppression may not be restricted against the dysplastic clone and may also affect nonclonal (normal) hematopoiesis. Lymphocyte-depleted long-term bone marrow cultures from patients with lower risk MDS also



generated some nonclonal hematopoietic colony growth, which was abrogated when T lymphocytes were present in the culture system [82]. Autologous lymphocytes were particularly cytotoxic in patients with hypoplastic MDS, trisomy 8, or bearing the DR15 allele [84]. The suppressive role of autologous T lymphocytes has been very nicely demonstrated in a patient with MDS and cyclic hematopoiesis, in whom, the percentage of marrow CD3+ lymphocytes was inversely correlated with neutrophil and platelet count during the various phases of ineffective hematopoiesis [85].



**Figure 1.** Correlation of AMLR to APLR in each subject tested with MLRs. By correlating AMLR to APLR an almost complete, discrete compartmentalization of the three main subject groups was found. Normal controls exhibited higher AMLR than APLR, patients with lower risk MDS had lower, both types of MLRs, whereas patients with an excess of marrow blasts showed impaired AMLR and very increased APLR.

#### 6.4. The role of clonal hematopoietic cells in the induction of immune dysregulation

The strong MLR against autologous clonal CD34+ progenitors observed in patients with RAEB was inversely correlated with marrow cellularity. All four patients with a strong APLR had marrow cellularity of <35% and marrow blast cell percentage of 5–10% (RAEB-1). These patients, although severely pancytopenic and transfusion dependent, had a delayed evolution to AML or they did not progressed at all. In two of them who progressed, marrow cellularity was increased and in a new APLR, performed at the time of AML progression, lymphocyte activation against autologous blasts had been abrogated [86]. Thus, it appears that lymphocyte

activation in patients with RAEB is rather inversely correlated with leukemic burden, but immunologic memory for this reaction is maintained and patients who lose their immune activation during leukemic transformation may regain it following the achievement of remission. Moreover, maintenance of remission is completely dependent on the presence of autologous cytotoxicity against leukemic cells, mainly exerted by NK cells. This cytotoxic reaction is disappeared upon relapse of the leukemia [87]. Unfortunately, immune activation against clonal cells is not the rule and T cells in MDS (particularly  $T_{\gamma\delta}$  cells) may respond poorly, if not at all, even following IL-2 stimulation, despite normal IL-2R expression, demonstrating impaired immune surveillance against the clone [51]. Indeed, the Czech group did not find any significant lymphocyte activation in eight out of nine patients tested and confirmed the nonclonal origin of the T cells [71].

Clonal dendritic cells can also induce T-cell stimulation in AML. Proliferative reaction against these cells is high but results in the generation of cytotoxic T lymphocytes with low activity against autologous or allogeneic nonleukemic targets [88]. Dendritic cells of MDS patients in Allo-MLR systems are poor stimulators for both normal and MDS-derived T cells, indicating an impaired antigen presenting capacity. These cells, generated from CD34+ cells, although immunophenotypically normal, were significantly decreased as were also the populations or circulating myeloid- and plasmacytoid-derived dendritic cells, confirming ineffective “dendritopoiesis” [89] and produce less IL-12 and more IL-10 in response to LPS and IFN- $\gamma$  showing qualitative and quantitative defects of cytokine production.

Blast cells exert direct suppressor activity on the activation, TH1 polarization and proliferation of T lymphocytes. This activity is mediated through protein substances transcribed via the NF-AT and NF- $\kappa$ B signals by inhibition of transition from  $G_0$  to  $G_1$  phase [90]. Upon NK cells, leukemic cells induce impaired killing capacity, reduced TNF- $\alpha$  and IFN- $\gamma$  production, reduced CD107 $\alpha$  degranulation, downregulation of the NKp46- and upregulation of the NKG2A receptor expression, effects directed via IL-10, and favoring clonal escape and expansion [91]. In other instances, however, blast cells induce lymphocyte activation, as previously described, with the production of IL-2, IL-4, IL-10, IL-13, and IFN- $\gamma$ . Lymphocyte culture supernatants, when further activated with IL-2, generate strong cytotoxic LAK and NK cells inducing lysis of autologous and allogeneic leukemic cells [92]. In the majority of cases, immune effector function of NK-T and NK cells, observed in some patients with MDS, are abolished on leukemic transformation.

## 7. Immunopathogenetic aspects of myelodysplastic syndromes

From the pathogenetic point of view it appears that an initial, harmful event (viral, drug, irradiation, etc.), affecting the pluripotent hematopoietic stem cell compartment in the bone marrow, may antigenically alter a minor population of these cells. Even when the consequences of the harmful event are negligible and maturation and differentiation processes might remain almost intact, it is possible that an immunologic reaction could be initiated. This reaction is directed against the even minimally modulated hematopoietic progenitor cell

population. Indeed, the strength of the autologous cytotoxic immune reaction, frequently accompanying the emergence of a dysplastic clone, is not related to the complexity/severity of the cytogenetic abnormalities, and a minor genetic damage may induce a strong reaction. Conversely, complex chromosomal aberrations and other gross genetic damage, leading to hematopoietic failure, may induce a weak or not any immune reaction. This reaction may be less specific and may also generate cytotoxicity, not only against the affected cells, but also to the unaffected/normal hematopoietic progenitors, inducing apoptosis and resulting in stem-cell depletion and hematopoietic failure. Soluble factors (cytokines) released by the activated lymphocytes might also harm accessory/stromal cells. This cascade of events usually leads to aplastic anemia. When the initial harmful event induces deeper genetic damage in a pluripotent stem cell and this cell, although genetically altered, succeeds in escaping from apoptotic cell death may generate an abnormal (dysplastic) clone. Clonal cells continue to trigger the immunocompetent cells, but the latter although activated cannot eliminate the clonal cells, which continue to escape, gradually expand, and suppress the unaffected/normal stem cell compartment through at least two mechanisms:

- (1) Immune effector cytotoxic cells can destroy more easily the nonclonal/normal progenitor cells as a result of clonal cell escape from the immune attack.
- (2) Secondary genetic alterations occurring gradually provide growth advantage to the clonal cells.

Thus, immune activation may perpetuate and when cytotoxic activity is ineffective and incapable to eliminate clonal cells, it becomes an “immunologic abnormality.” The more effective the immune activation, the higher the degree of apoptosis induced, affecting more and more marrow cellularity and creating a syndrome mostly similar to aplastic anemia. Therefore, the decreased marrow cellularity observed in some marrow failure syndromes might be considered an “adverse event” of an effective immune reaction capable to restrict the growth of the abnormal/mutated/genetically altered clonal cell population. On rare occasions, the intensive immune activation, augmented by an infection or a blood transfusion, may be capable to completely eradicate the dysplastic clone leading to spontaneous complete remission even after evolution to AML. In contrast, when immune activation is ineffective, clonal expansion and evolution continues unimpededly until the stage of AML. At this stage, either passively, due to high “antigenic burden,” or actively, through mechanisms, induced by the leukemic cells, immune tolerance or immune paralysis is established abrogating further immune reaction [93]. In rare instances, even after evolution, immune activation may be maintained and result in an oligoblastic/hypoplastic AML. Conversely, when immune activation is abolished early or when the dysplastic/neoplastic clone achieves in earning immune tolerance, the evolution might be uneventful and lead to a hypercellular AML.

About 10–15% of MDS patients at initial presentation have a hypoplastic marrow (cellularity  $\leq 30\%$ ). These patients exhibit more severe cytopenias, various degrees of trilineage dysplasia, more prominent immune abnormalities, and usually a normal karyotype or single chromosomal abnormalities. Although hypoplastic MDS share many similarities with aplastic anemia, different molecular mechanisms of marrow damage have been identified between them and

other/nonhypoplastic MDS. Among them development of oligoclonal expansion of cytotoxic T lymphocytes, overexpression of TRAIL- and Fas ligand-induced apoptosis, underexpression of Flice-like inhibitory protein long isoform (FLIPL), and increased production of IFN- $\gamma$  and TNF- $\alpha$  are included. Patients with hypoplastic MDS have more stable clinical course and lower evolution rates in relation to patients with nonhypoplastic disease of the same FAB/WHO categories. They show good response to treatment with corticosteroids, cyclosporine-A, antithymocyte globulin, or alemtuzumab, and to various combinations of the above. Overall survival varies and if patients will not succumb to a severe infection, they may retain a prolonged leukemia-free survival [94, 95].

Suppressor/cytotoxic autoimmune reactions are more frequently identified among lower risk MDS, have specificity against the pluripotent or an early committed, usually erythropoietic progenitor cell, and are associated with higher degree of marrow apoptosis. In the majority of cases, the autoimmune process includes the production of specific antierythroblastic antibodies without the positivity of direct antiglobulin test. The production of such IgG autoantibodies can be provoked *ex vivo* following antigenic stimulation [96]. These patients show higher caspase-3 activity and lower TNF- $\alpha$  and IL-4 production. Analysis of the total IgM and IgG antibody repertoire in 10 MDS patients without prominent autoimmune disease or known autoantibody and in 10 healthy controls revealed different patterns of antibodies against self-antigens in MDS patients from those of controls, and patterns of IgG antibodies had distinct profiles implying disturbed self-recognition related to pathogenetic mechanisms of the disease [97].

Increased marrow apoptosis is a dominant feature of MDS and affects all hematopoietic cell compartments from the more immature-undifferentiated to the mature and recognizable cells. The apoptotic rate of CD34+ cells in normal subjects has been calculated at about 1%, whereas in MDS, it ranges from 3 to 15%. Higher apoptotic rate is usually found in patients with early MDS and in few patients with an excess of blasts [98]. Apoptotic rate may vary in the same patient at different time points reflecting also the evolution of mutational status of the clonal cells. Specific cytogenetic abnormalities, such as trisomy 8 have been associated with higher degree of apoptosis. Apoptosis is a multifactorial process in MDS with a possible contribution of the immune effector cells. Clonal cells' death could create abnormal structures with potentially (auto)antigenic properties and apoptosis can represent the causative factor of the initiation of autologous cytotoxic immune reaction. Indeed, increased apoptotic cell rate has been associated with higher marrow cytotoxic T-cell infiltration and in many instances by oligoclonal T cells in MDS patients, who also express the TIA-1 antigen on their hematopoietic cells [99]. The proapoptotic marrow microenvironment triggers stromal cells to produce IL-32, which in turn induces further TNF- $\alpha$  transcription, thus establishing a vicious cycle. IL-32 expression has been found many folds higher in the stromal cells of MDS patients, rendering this cytokine a specific stromal cell marker for MDS [100].

Although immune activation plays the dominant pathogenetic role for the generation of marrow failure in aplastic anemia, in MDS this cannot be easily identified in every individual patient. In other words, it is not identifiable which part of the hematopoietic failure results from the clonal disorder *per se* and which is attributed to the immune activation. This fact could



explain, at least in part, why there is not a uniform response rate to the immunosuppressive treatment and this rate may vary widely in different series of patients, irrespective of FAB or WHO category, cytogenetics, and the severity of the cytopenias [101].

## 8. Immunosuppressive/immunomodulating treatment applied to patients with MDS

Corticosteroids are the most widely used immunosuppressive treatment administered to patients with MDS and autoimmune diseases [17, 20]. Response rates vary broadly and the required dose depends on the type of autoimmune disease, MDS subtype, chronicity of the condition, and other factors. Although symptom resolution may be fast, autoimmune disease may relapse during tapering, demanding higher doses, which may not be tolerable by elderly patients. Thus, corticosteroids usually lead to partial or transient response and second-line treatment with other agents is necessary. Steroids may also benefit hematopoiesis, improving cytopenias and reducing RBC transfusion needs. Responses are mainly seen in patients with lower risk IPSS, with a specific profile, but also by some patients with RAEB [102] and may be long-lasting and maintained with small maintenance steroid doses.

Cyclosporin-A (Cy-A) is the second more widely used immunosuppressive agent and has also been used in combination with cytotoxic chemotherapy as a modulator of multidrug resistance, which is commonly found in higher risk MDS and in AML following MDS. Cy-A is effective even at lower doses, aiming to achieve serum levels lower than those desirable in aplastic anemia and in allogeneic stem cell transplantation and therefore is well-tolerated and induces durable remissions [103, 104]. Retrospective evaluation of 50 patients showed a hematological improvement and particularly an erythroid response in 60%. Better response was achieved by patients with hypoplastic marrow, favorable karyotype, or carrying the DRB1\*1501 allele [105]. The NIH group has reported more frequent expression of the HLA-DR2/HLA-DR15 allele in patients with MDS and aplastic anemia compared to a control population and an association of the expression of this allele with a favorable response to immunosuppression [106]. Cy-A added to T-lymphocyte cultures decreases IFN- $\gamma$ - but not Fas-L production and lead to abrogation of the inhibitory activity of the supernatant on hematopoietic colony formation. However, the growth of secondary colonies continues to be decreased due to low number of pluripotent CD34+ progenitors.

Probably the most effective immunosuppressive treatment is antithymocyte globulin (ATG), which has been given to MDS patients with any marrow cellularity [101, 104]. Hematopoietic improvement is achieved following elimination of the autoreactive cytotoxic T cells and may result in restoration of the dysplastic marrow and peripheral blood morphology. In many instances cytogenetic complete remission has also been reported, whereas in others, hematologic remission is not accompanied by cytogenetic remission. In these cases, most probably immune activation mainly suppresses nonclonal hematopoiesis without significantly disturbing the dysplastic clone. Finally, rapid evolution to AML or increase of marrow blasts despite hematological improvement has occasionally been reported following treatment with Cy-A or



ATG [107]. In these cases, immune activation may effectively suppress clonal cells and its abrogation has favored the unimpeded clonal expansion and evolution. Mofetil mycophenolate (MMF) or alemtuzumab can be used when corticosteroids and/or Cy-A are ineffective or contraindicated, or when severe adverse effects emerge, but the experience with these agents is limited. The main drawback of immunosuppression is that combined with the usually coexisting neutropenia substantially increases the risk for common and opportunistic infections, even when all prophylactic measures are applied. Cy-A, in particular, may further impair previously existed renal failure and may induce various adverse events as a result of pharmacodynamic interactions to patients concomitantly treated with many other drugs.

Immune-stimulating treatment, targeting NK-/NK-T cells and aiming to generate cytotoxic T-cell activity and eliminate the dysplastic clone, has been associated with rather disappointing results. A promising message is that newer immunomodulating drugs, such as lenalidomide, appear to increase NK T cells and improve their function, including cytokine production, although this is not the major mechanism of action of the drug [108]. Hypomethylating agents, currently used particularly in higher risk patients, when effective and leading to complete response may also benefit autoimmune or hyperimmune clinical syndromes associated with MDS. It has also been suggested that 5-azacytidine has an independent immune-modulating activity and that remissions of the auto-/hyperimmune syndrome may occur independently of the induction of hematological and cytogenetic response, and might also be effective in cases in which other immunosuppressive treatments have been proved ineffective [109].

The injudicious use of immunosuppressive treatment in MDS may become a trench knife [110]. Patients exhibiting overactive immune response, but clonal hematopoiesis, even when sharing a hypoplastic bone marrow, might need even more effective immune activation to wear down the dysplastic clone. Similarly, patients with an established clonal disease, but without any immune activation, could potentially gain benefit with the administration of immune stimulation in an effort to eliminate the clone. On the other hand, abrogation of an overactive immune stimulation should be attempted when this activation suppresses primarily the residual normal/nonclonal hematopoiesis and minimally disturbs the development of the abnormal/dysplastic clone. When immune activation/reaction status cannot be identified and/or quantified, in the middle of established dysplastic hematopoiesis, a course of moderately strong immunosuppressive treatment with corticosteroids and/or cyclosporine could be administered, and in cases of a favorable response, careful tapering of the drugs should be tested in an effort to maintain the obtained response.

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