Review

Myelodysplasia-associated autoimmunity: clinical and pathophysiologic concepts

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Abstract

Myelodysplastic syndrome (MDS), an acquired clonal disorder of haemopoietic progenitor cells, is characterized by haemopoietic insufficiency associated with cytopenias, leading to serious morbidity plus the additional risk of leukaemic transformation. In MDS an acquired insult to the haemopoietic stem cell leads to impaired differentiation and myelodysplasia. However, there is increasing evidence that the marrow failure of MDS is immune-mediated. A model of MDS pathophysiology suggests that transformation of normal stem cells induces an autoimmune T-cell response with the bone marrow as the target organ. This autoimmune attack results in chronic overproduction of pro-apoptotic cytokines, especially tumour necrosis factor alpha (TNFα). In addition, several reports have revealed that approximately 10% of MDS patients have clinical autoimmune disorders. This review illustrates the cellular/molecular mechanisms and the implication of the tumour suppressor gene interferon regulatory factor-1 (IRF-1) in the pathophysiology of MDS-associated autoimmune deregulation.

Keywords Apoptosis, autoimmune manifestations, bone marrow failure, ineffective haemopoiesis, IRF-1, myelodysplastic syndrome.


Introduction

Primary myelodysplastic syndromes (MDS) are heterogeneous clonal haemopoietic stem-cell disorders, characterized by ineffective and dysplastic haemopoiesis with a varying degree of peripheral cytopenias and an increased probability of developing acute leukaemia. The natural history of these syndromes ranges from more indolent forms of disease spanning years to those with a rapid evolution to acute myeloid leukaemia (AML). Precise pathogenesis of MDS is as yet unknown, but it has been generally suggested that MDS arise from a haemopoietic stem cell harbouring irreversible DNA damage. Although MDS encompass a heterogeneous group of diseases, dysplastic cellular morphology and bone marrow failure constitute common underlying aspects beyond its clinical diversity. Despite recent scientific advances the exact role of immune system deregulation in the pathogenesis of myelodysplasia-associated bone marrow failure remains poorly defined. On the other hand a distinct subset of MDS patients manifest overt autoimmune manifestations (AIMs); the underlying pathogenesis and prognostic significance of which remain controversial (Fig. 1). This review will briefly highlight the mechanisms of autoimmune-mediated myelosuppression in MDS and the pathophysiology of immune deregulation in preleukaemic states.

Role of apoptosis in the pathophysiology of ineffective haemopoiesis-associated myelodysplasia

One characteristic feature of MDS is ineffective haemopoiesis, a condition in which cellular bone marrow is unable to produce and deliver adequate numbers of mature cells to the peripheral blood. Increased cellular proliferation, along with exaggerated programmed cell death (apoptosis),
Razapaska et al. detected a high proportion of CD34+ cells with sub-G1 DNA in the early stages of MDS compared with advanced MDS, and AML patients and normal individuals [6]. As the sub-G1 DNA fraction correlates with the levels of low-molecular weight fragmented DNA, which is the biochemical signature of apoptosis, the authors suggested that apoptosis was significantly increased within the CD34+ cell in early MDS patients. Studies in which apoptosis was assessed by annexin V binding came up with analogous results. Parker et al. showed that the numbers of apoptotic CD34+ cells appeared to inversely correlate with the prognostic stage, proposing that patients with evidence of increased apoptosis may have better outcomes [8]. Conversely, some investigators have demonstrated that CD34+ cells from patients with MDS show resistance to apoptosis, while others correlate ineffective erythropoiesis with an increase in intramedullary apoptosis of differentiated cells and to a lesser extent immature CD34+ cells [4,10]. Despite the fact that the precise phenotype of cells undergoing apoptosis in MDS has not yet been clearly defined, a clear trend favouring apoptosis of early haemopoietic progenitors has emerged [5,6,8,11]. Moreover, the concept of the high propensity of immature progenitors to undergo apoptosis in early stage MDS led to the hypothesis that MDS progression arises through multiple hits that alter levels of CD34+ cell apoptosis, enabling the proliferation of these primitive cells [12].

Excessive apoptosis in MDS marrow cells has been ascribed to abnormalities of growth and differentiation intrinsic to the myelodysplastic clonal population. It was hypothesized that causative genomic lesions gives rise to defective cell-cycle machinery and DNA repair effectors, leading to abnormalities in the apoptotic control [13–15]. However, recent findings emphasize the role of extrinsic factors, which compound the intrinsic stem-cell defect contributing to the pancytopenia and possibly to leukaemic progression. Cell death might be triggered by the bone marrow microenvironment either through a relative deficiency of growth factors or an overproduction of cytokines inducing cell death. Alterations of the delicate balance between growth stimulatory and inhibitory cytokines within the MDS microenvironment have been the focus of a large number of studies. Interleukin-1β (IL-1β), IL-6, IL-8, stem-cell factor, granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin and transforming growth factor-β (TGF-β) have been measured in the serum or marrow of MDS patients with various, even conflicting, results [16–18]. However, several studies have concluded that increased tumour necrosis factor α (TNFα) levels in the marrow microenvironment, high Fas antigen expression on the CD34+ cells and the resultant activation of apoptosis generating proteases (caspases), especially caspase 3, are the cardinal events leading to the apoptotic death of marrow cells in MDS [19–22].

Several independent groups have reported elevated levels of TNFα mRNA and protein in the bone marrow of MDS patients, correlating with raised rates of apoptosis [23–27]. Patients with MDS with severe or transfusion-dependent anaemia display increased serum levels of TNFα [28,29].
In accordance with these findings, suppression of TNFα, either by pentoxifylline or anti-TNFα antibody, resulted in a reduction of apoptosis, enhanced in vitro haemopoietic clonal culture and a favourable clinical response in some patients [25,30]. The high TNFα expression has been implicated in the increased free radical production observed in CD34+ marrow cells with resultant oxidative damage and cell death [31]. Moreover, both in vivo and in vitro studies have demonstrated that the Fas/Fas ligand (FasL) system performs a critical function in producing apoptosis in several organ systems after triggering of Fas by FasL [32]. Molecular crosslinking of Fas by its natural ligand or by agonistic antibodies results in the sequential triggering of caspases whose activation is required for the propagation of the biochemical events responsible for induction of apoptotic cell death [33,34]. Primitive haemopoietic progenitor cells express low amounts of Fas [35]. However, different cytokines have been shown to up-regulate Fas expression on CD34+ cells, including TNFα and interferon γ (IFNγ) [24,36]. As Fas is overexpressed on MDS CD34+, it has been hypothesized that TNFα prime bone marrow CD34+ cells for Fas-induced apoptosis, suggesting a Fas–FasL interaction as a possible pathogenetic mechanism contributing to immune destruction of CD34+ cells in human myelodysplasia [10].

There is now much evidence that increased haemopoietic progenitor cell apoptosis contributes to the ineffective haemopoiesis and peripheral cytopenias, at least in the earliest stages of MDS. As attractive as it is, the hypothesis that excessive apoptosis has an essential physiopathologic importance in the bone marrow failure of myelodysplasia has been criticized for the absence of convincing answers to crucial questions. How such accelerated apoptosis is initiated or acquired is not yet understood. Whether a molecular defect giving rise to increased apoptosis is a part of clonal abnormalities inherent to myelodysplasia it has not yet been elucidated. Either central to MDS or an epigenetic abnormality, the apoptotic propensity of the dysplastic marrow is an important biological feature of the disease and research of the aberrant cytokine production or the abnormal marrow microenvironment may provide important therapeutic targets [37]. Since the first reports demonstrating defective bone marrow stroma in patients with aplastic anaemia, the participation of marrow stroma in the MDS pathology is an area of active investigation [2,38]. Apoptosis of the MDS stromal cells has been demonstrated, along with their decreased capacity to support normal haemopoiesis. Myelodysplastic syndrome-derived stromal cells were found to induce apoptosis of normal CD34+ cells, while long-term bone marrow cultures of MDS patients are characterized by decreased or absent stromal growth [39]. Long-term marrow cultures, which provide an in vitro model of the haematopoietic microenvironment, are complex, containing a mixture of cells and factors critical for the regulation of haematopoiesis. In an effort to functionally dissect the haematopoietic microenvironment, several groups have established cloned stromal cell lines that identify individual microenvironment components. Therefore, the heterogeneity of stroma function detected among MDS patients appears to be related to the cellular composition of the bone marrow stroma, which may differ from patient to patient and from patient to control. As no cloned stromal cell lines are used, there is increasing evidence that the trafficking and homing of autoreactive T lymphocytes in the bone marrow may affect the haemopoietic-supporting capacity of stroma through abnormally produced cytokines.

While the adhesive interactions between haemopoietic cells and the underlying marrow stroma remain a field relatively unexplored, cytokine production from marrow stroma, specifically TNFα production, is another subject of controversy. Both stromal and nonstromal cells have been implicated in aberrant cytokine production but as we failed to precisely identify the cellular origin of these factors the fundamental biologic abnormality leading to the pro-apoptotic environment in MDS marrow is as yet unknown [40]. The question concerning the correlation between the apoptotic propensities of the dysplastic marrow and the enhanced ability of the myelodysplastic clone for leukaemic transformation is even more challenging. Important in this context is the determination of which cells, residual-normal cells or aberrant and possibly clonal malignant cells, are most affected within the pro-apoptotic environment. Very few studies have addressed the question of whether apoptotic cells are predominately clonal or not. Bogdanovic et al. found that the apoptotic index in patients with karyotypic changes was no higher than in patients with a normal karyotype, in agreement with Parker et al. who found no correlation of annexin V positivity and cytogenetic abnormalities [12,41]. Raza et al. found that leukaemic blasts from bone marrows of advanced stage MDS patients were negative for apoptosis [30]. The existing evidence suggests that resistance to apoptosis is one of the characteristic features of the abnormal leukaemic clone probably leading to its final domination in the dysplastic marrow. The underlying mechanisms contributing to this behaviour remain obscure. Anti-apoptotic genes, such as Bcl-2, are overexpressed in relation to pro-apoptotic members of Bcl-2 family (Bad, Bax) and c-myc in late MDS [6,12]. Loss of susceptibility to Fas-mediated apoptosis in MDS patients progressing toward a leukaemic phase has also been reported, but all these seem to represent mechanistic changes rather than pathogenetic events [42].

The acquisition of secondary genetic events is a model proposed to explain the stepwise progression of MDS to acute leukaemia. These yet unrecognized epigenetic hits possibly perturb apoptotic cell-signal transduction pathways of the primitive clone, altering its apoptotic nature and offering it a growth or survival advantage. Alternatively, excessive apoptosis in early stage MDS could be interpreted as an immune response to antigens expressed by the clonal aberrant cells. As will be further discussed below, a T-cell attack on haemopoietic cells has been documented in MDS patients [43]. The ‘T against the Clone’ scenario includes an immune reaction operated by T cells, which in early stage MDS creates an apoptotic environment by the release of inhibitory cytokines and up-regulation of Fas expression on haemopoietic progenitors. This process probably delays the leukaemic evolution, but also leads to bone marrow failure.
Suppress autologous bone marrow CFU-GMs cells peripherally lymphocytes from MDS patients could be mediated [46,47]. Indeed Smith et al. first showed that pancytopenia in MDS may be in part lymphocyte-mediated [46,47], led to the hypothesis that cytopenia in MDS may be in part lymphocyte-mediated [46,47]. As the disease progresses the immune system becomes ineffective to control the accumulatively damaged myeloid clone, thus allowing its expansion. CD8 CTL, CD8+ T-cytotoxic cells, IRF-1: interferon regulatory factor-1.

As the disease progresses immune reactions become ineffective in controlling the accumulatively damaged myeloid clone, thus allowing its expansion (Fig. 2). Studies reporting increased Fas susceptibility and apoptosis of MDS cells with trisomy 8, but not with monosomy 7, support this perception [44,45]. In contrast, the autologous immune response against leukaemic clones in patients with AML and chronic myeloid leukaemia is less evident. The reasons for this failure include: insufficient antigen presentation by the malignant cells, immune suppression by soluble or cellular factor(s), and an insufficient number of specific lymphoid cells to react with the rapidly growing clone.

**Immune pathophysiology of myelodysplastic syndromes**

Immune-mediator cells, particularly T cells are part of the haemopoietic microenvironment, which regulates both proliferation and differentiation of haemopoiesis. The haematological response of some pancytopenic MDS patients to antithymocyte globulin (ATG), a polyclonal antibody with direct and indirect lymphocytotoxicity, led to the hypothesis that pancytopenia in MDS may be in part lymphocyte-mediated [46,47]. Indeed Smith et al. first showed that peripheral blood lymphocytes from MDS patients could suppress autologous bone marrow CFU-GMs cells in vitro [48]. On studying patients who had been treated with ATG, Molldrem et al. found that the removal of CD3+ or CD8+ T cells from bone-marrow progenitor cultures resulted in a significant increase in CFU-GMs in responders to ATG, but not in nonresponders [49]. Since then, T-cell-mediated marrow inhibition in MDS patients has been demonstrated in several studies, whereas important questions have been raised concerning the role of these autoreactive lymphocytes in the etiology of cytopenia in MDS.

MDS and autoimmunity

MDS patients exhibit high percentages of CD8+, CD28– CD57+ cells, a phenotype characteristic of high cytotoxic cells (CTL), consistent with mature effectors’ function [50,51]. In many infectious and autoimmune disorders such cytotoxic cells have been found to differentiate either from naive or proliferating memory cells, under antigen-specific T-cell receptor (TCR) triggering and costimulatory signals. Further analysis of these activated T cells by spectratyping (assessment of the variable portion of the beta chains of the TCR loci; TCR VB-chain repertoire) revealed limited TCR representation, owing to the expansion of dominant T-cell clones [51–54]. Three independent groups studying MDS using either flow cytometry or CD3 analysis (spectratyping) observed overuse of individual TCR VB-chains with unique complementarity determining region-3 (CDR3), resulting in TCR VB repertoire skewing in MDS patients. This indicates the presence of a persisting clonally expanding T-cell population.

T-cell clonality in MDS was first interpreted as evidence of T-cell involvement in the stem-cell disorder [55]. Although studies on the clonal nature of lymphopoiesis in myelodysplasia have had ambiguous results, a growing number of surveys exploring the clonal-cell lineage involvement in myelodysplastic syndromes concluded that the progeny cell of origin was restricted to the myeloid lineage [56,57]. However, clonality of the entire T-cell population in these studies was performed by analyzing X-chromosome inactivation (XCI) patterns of peripheral blood cells and bone marrow progenitors from female MDS patients using the human androgen-receptor (HUMARA) assay. Spectratyping and XCI analysis are two entirely different techniques, analyzing two entirely different processes. When MDS lymphocytes are considered to be polyclonal or monoclonal based on their XCI patterns, three points need to be taken into consideration. First, the possibility still remains that, owing to their long life-span, polyclonal lymphocytes in MDS are part of a population originating before the onset of a primary mutation affecting a progenitor common to the myeloid and lymphoid lineages. Second, polyclonal peripheral blood lymphocytes do not exclude the presence of a minor monoclonal lymphoid population, which is selectively retained in the bone marrow. Furthermore, the entire haematopoiesis in patients with aplastic anaemia in remission may be of clonal origin using the XCI analysis without any signs of clonality in the TCR VB repertoire when examined by spectratyping or spectratype-related techniques [58]. This may either indicate that only small expansions of αβT cells have a pathophysiological role on aplastic anaemia or that the αβT cells are not distorting the stem-cell population but that γδT cells are involved [59,60].

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Persisting clonal expansions are not necessarily an expression of a malignant process; they also represent clonal or oligoclonal expansion of possibly autoreactive T cells and are most marked in CD8+ populations. The increase of CD57 expression by the CD8+ cells, as well as the demonstration of expanded TCR VB families expressing CD57, are characteristic features of diseases attributable to an activated immune environment such as acute graft vs. host disease, multiple sclerosis and rheumatoid arthritis [61–63]. In these well-established autoimmune disorders clonally expanded T cells bearing similar VB subfamilies proliferate dominantly in different patients, reflecting a specific response of auto-reactive T cells to certain antigens. The recent findings of expanded cytotoxic T-cell clones in MDS patients has led to the hypothesis that MDS may be the result of an autoimmune reaction directed against marrow-stem cells. Hypothetical models propose immune triggering of the effectors’ lymphocytes, either by aberrantly expressed oncogenes and fusion genes in the haematopoietic progenitor cells or by super-antigens derived from bacteria or viruses [52,64]. Such effector cells should have limited TCR usage among different MDS patients, reflecting the common pathogenic, antigen-driven oligoclonal expansion [61–63].

Despite recent facts provided by advanced studies we still lack incontestable proof of an antigen-driven T-cell response in the development of bone marrow failure in MDS, not to mention the causal antigen that elicits the T-cell attack against haematopoietic progenitor cells. Studying the TCR VB profiles of MDS patients treated with immunosuppressive agents, Epperson et al. found a repetitive pattern of VB β 23-Jβ 2·1 region usage confirming a nonrandom T-cell clonal expansion in these patients [52]. An analogous study of Kochenderf et al. revealed preferential use of the TCRVβ4 family within the clonally expanded T cells of seven MDS patients [65]. Whatever they are, the antigenic peptides leading to CTL selection in MDS are not known. However, many diseases have been classified as autoimmune even though the inciting antigen has never been identified. Therefore, the identification of the antigen does not require making the autoimmune hypothesis of MDS believable.

Matsutani et al. searched for common antigenic determinants by examining the nucleotide and amino acid sequences of the CDR3 of TCRα and β chains of expanded T cells isolated from the peripheral blood of myelodysplastic patients with erythroid hypoplasia [53]. The gene segments of the variable (V) and joining (J) regions varied among these patients, while the deduced amino acid sequences of CDR3 were also heterogeneous, without revealing a common motif. Studies using HLA-peptide tetramers and analyses of crystal structures of TCR-peptide-MHC complexes suggest that the TCR makes only few contacts with the CDR3 region and the peptide in the MHC groove, and that other CDR3 residues contact the MHC alpha helices. Therefore, the heterogeneity of the C3D sequence may be owing to the difference in HLA molecules among the MDS patients; however, antigen-dependent proliferation cannot be excluded.

**Myelodysplastic syndrome and aplastic anaemia: possible pathogenetic links**

While MDS is a distinct pathophysiological entity from aplastic anaemia (AA), hypoplastic MDS, which is characterized by cytopenias, bone marrow dysplasia, and marrow hypopcellularity, has been difficult to distinguish from AA. The pathophysiology of the cytopenia associated with marrow failure in the two conditions, specifically the T-cell-mediated immune suppression of haemopoiesis, may be similar or even identical [43]. Myelodysplastic syndrome shares some of the features of acquired AA, a disease with an established autoimmune pedigree. Both in AA and MDS, plasma TNFα and IFN-γ levels are high and T-cell-mediated myelosuppression occurs [66,67]. Abnormal CD4 : CD8 ratios, increased activated cytotoxic T cells, as demonstrated by a higher percentage of CD8+ CD28− and CD8+ CD28−CD57+ cells, and skewing of the TCR VB CDR3 patterns have been detected in both diseases suggesting an autoimmune T-cell-mediated myelosuppression in both [43,68]. The incidence of T-cell-mediated marrow suppression in MDS is not known. It is of interest that the T-cell repertoire was mainly explored in patients with predominately hypoplastic marrows treated with immunosuppressive agents, thus suggesting a selection bias. As MDS are both clinically and pathogenetically polymorphic diseases the study of unselected patients may produce varying results, proving difficult to interpret. Although it cannot be excluded that these clonally expanded T cells contribute to suppression of haemopoiesis, we should consider with caution the hypothesis that the antigen driven T-cell expansion is an essential component of the bone marrow failure in the general MDS population. Alternatively bone marrow failure in MDS patients may derive from polyclonal T-cell activation leading to excessive production of myelosuppressive cytokines or delivering death signals via the Fas/Fasl system. Reports documenting predominance of the polyclonal T-cell populations in aplastic anaemia further justify this scepticism [69].

**Autoimmune phenomena in patients with myelodysplastic syndromes**

While a high percentage of researchers subscribe to the popular concept of immune system involvement in the pathogenesis of MDS, the stronger evidence favouring this theory is a peculiarity in the clinical picture of these disorders, i.e. the substantial number of MDS patients manifesting overt autoimmune disorders [70–73]. The observed autoimmune phenomena are various and its specificity is not clear. They are probably initiated and perpetuated through nonspecific alterations of the immune system in MDS, such as overproduction of certain kinds of proinflammatory and/or immunoregulatory cytokines. However, it is not clear how these autoimmune phenomena could have any pathophysiological link with the immune response against the MDS clone, as most of the target tissues are nonhaematopoietic.
Furthermore, the clinical response of these manifestations and occasionally the simultaneous dramatic haematological improvement to immunosuppressive therapy is not indicative of a common pathogenetic origin.

This association of myelodysplasia and immunologic abnormality disorders was first reviewed by Hamblin who described two cases of autoimmune haemolytic anaemia in 104 patients with MDS [70]. Sporadic case reports and several retrospective studies have further highlighted the association. An estimated 10% incidence of AIMs in MDS was reported in these studies [71–73]. A large Japanese single institution study of 153 MDS patients revealed that 12% developed AIMs [74]. Billstrom et al. identified a similar incidence, and Castro et al. also reported 10% of MDS patients displaying rheumatic manifestations [72,73]. Such phenomena may include an acute systemic vasculitic syndrome, skin vasculitis, fever, arthritis, pulmonary infiltrates, peripheral polyneuropathy, glomerulonephritis, and even classical connective tissue disorders, such as relapsing polychondritis (Table 1). In addition, asymptomatic immunological abnormalities, first recognized by Mufti et al., have also been reported in these patients [75]. Since then, research has focused on such abnormalities underlying or predisposing myelodysplasia. On investigation, the immune system dysfunction was found to include decreased NK cell activity, impaired antibody-dependent cell killing, diminished CD4 cell numbers, and for B-cell function altered immunoglobulin levels, monoclonal gammapathies, and various autoantibodies [70,76]. In the Japanese cohort, 63% displayed some abnormalities in the immunological tests: hypergammaglobulinaemia, the presence of antinuclear antibodies, rheumatoid factor, anti-DNA antibodies, and a positive Coombs’ test [74].

Among 70 MDS patients diagnosed at our Department of Pathophysiology, Medical School of Athens, over a period of 4 years, 13 MDS patients developed AIMS: seven male and six female with ages ranging from 51 to 80 years. The estimated prevalence of AIMS in our cohort of MDS patients was 18.5% [77]. We classified our patients according to the FAB classification and International Prognostic Scoring System (IPSS) (Table 2) [78,79]. Clonal abnormalities were identified in six patients and included 5q-, +8, −5, −7, deletion 20q, and deletion 3q. All AIMS included the following categories: systemic vasculitis, skin vasculitis, isolated autoimmune manifestations such as autoimmune thrombocytopenia, polyarthritis and colonic ulcerations, and classic connective tissue disorders such as relapsing polychondritis and Sjogren’s syndrome. The majority of our patients manifested vasculitic overlap syndromes, with leukocytoclastic vasculitis being the most common manifestation. Two of our patients developed acute vasculitic syndrome, manifested with a sudden onset of fever, leukocytoclastic vasculitis, pleural effusions, non-infectious pulmonary infiltrates and peripheral neuropathy. Serological abnormalities included hyper/hypogammaglobulinaemia, direct Coombs’ test positivity, monoclonal paraproteinaemia, antimitochondrial autoantibodies, auto-antibodies against thyroid peroxidase and thyreoglobulin, antinuclear antibodies, and the presence of rheumatoid factor. Evidence of autoimmune disease prior to the diagnosis of MDS was found in four patients; MDS antedated AIMS in one patient and the remainder developed AIMS during the course of the haematological disorder. Autoimmune manifestations, which were presented prior to the diagnosis of myelodysplasia, included symmetric polyarthritis, temporal arteritis, glomerulonephritis and Sjogren’s syndrome, respectively. There was no association between MDS therapy and the onset of AIMS. We can conclude from the above study that the autoimmune manifestations are more common in MDS patients than previously thought. We now have to consider the possibility of an underlying MDS in patients with systemic vasculitis or other rheumatologic disorder, specifically in those who display an impaired haematological profile, unrelated to immunosuppressive or cytotoxic therapy.

### Table 1 Autoimmune disorders associated with myelodysplasia

<table>
<thead>
<tr>
<th>Autoimmune manifestations</th>
<th>Systemic autoimmune manifestations</th>
<th>Connective tissue disorders</th>
<th>Autoimmune cytopenias</th>
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<tbody>
<tr>
<td>Acute systemic vasculitis</td>
<td>Chronic autoimmune manifestations</td>
<td>Skin vasculitis, arthritis, glomerulonephritis, peripheral polyneuropathy</td>
<td>Autoimmune cytopenias</td>
</tr>
<tr>
<td>Chronic autoimmune manifestations</td>
<td>Connective tissue disorders</td>
<td>Systemic lupus erythematosus, relapsing polychondritis, Sjogren’s syndrome</td>
<td>Raynaud’s phenomenon</td>
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<td>Autoimmune manifestations</td>
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### Table 2 Haematologic data of myelodysplastic patients with autoimmune manifestations

<table>
<thead>
<tr>
<th>No of patients</th>
<th>FAB</th>
<th>Hg (g dL$^{-1}$) range</th>
<th>WBC ($\times 10^3$/mm$^3$) range</th>
<th>PLT ($\times 10^3$/mm$^3$) range</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>RA</td>
<td>7.6–13</td>
<td>2.1–5.3</td>
<td>10–192</td>
</tr>
<tr>
<td>2</td>
<td>RAEB</td>
<td>5.6–8</td>
<td>1.9–3.9</td>
<td>17–57</td>
</tr>
<tr>
<td>2</td>
<td>RAEB-T</td>
<td>7.5–9.7</td>
<td>1.5–3</td>
<td>15–80</td>
</tr>
<tr>
<td>2</td>
<td>CMML</td>
<td>9.3–9.9</td>
<td>7.3–13.7</td>
<td>65–90</td>
</tr>
<tr>
<td>1</td>
<td>RARS</td>
<td>9.4</td>
<td>4.8</td>
<td>100</td>
</tr>
</tbody>
</table>

FAB, French American British classification; Hb, haemoglobin; WBCs, white blood cells; PLT, platelets; RA, refractory anaemia; RARS, refractory anaemia with ring sideroblasts; RAEB, refractory anaemia with excess blasts; RAEB-T, refractory anaemia with excess blasts in transformation; CMML, chronic myelomonocytic leukaemia.
Accelerated exon skipping of IRF-1 mRNA in human myelodysplasia; a role for IRF-1 in autoimmune manifestations associated with myelodysplastic syndrome

The pathogenetic abnormalities that may cause secondary rheumatic features in MDS have not been clarified but it is unlikely that they are simply coincidental. Deregulation of cellular and humoral immune functions in MDS as reported above has been abrogated as the cause of the rheumatic diseases, yet this is merely the tip of the iceberg [70]. Abnormal T-cell responses to antigen presentation and abnormal B-cell and T-cell interactions particularly may be important in the pathogenesis of immune deregulation predisposing the development of autoimmune disorders [70]. In this regard, an overactive antigenic presentation resulting from a disordered monocyte/macrophage function leads to persistent immune stimulation and up-regulated cytokine secretion, allowing the activation of inflammatory mediators [70]. However feasible this theory may appear, we still have no conclusive evidence to this effect, as the exact pathophysiology of immune deregulation in myelodysplasia is as yet unknown.

Interferon regulatory factor-1 (IRF-1), originally identified as an IFNβ-promoter binding transcription factor, has been implicated as a mediator for IFN signalling, when induced by various stimuli such as viral infections, IFNs, retinoid acids and TNFα [80,81]. Moreover, IRF-1 functions as a tumour suppressor gene and plays an essential role in cell-growth control and surveillance against malignant development [82,83] (Fig. 3). Deletions and somatic mutations of IRF-1 gene have been implicated in the multiphase process of human myelodysplasia and leukaemic evolution [84]. Furthermore, alternative splicing of the IRF-1 mRNA resulting in truncated nonfunctional transcripts has been frequently observed in patients with MDS. Accelerated exon skipping of IRF-1 can therefore be considered as a possible mechanism of functional inactivation of IRF-1 in myelodysplasia [85]. Thus, loss of IRF-1 function may be a critical event in the development of human leukaemia.

Beyond its tumour suppressor function, IRF-1 regulates multiple stages of the immune response. It is a principle mediator for the innate immune system, being related to T-cell maturation in the thymic environment and the regulation of the IL-15 gene expression that is vital for survival and proliferation of NK cells in vivo [86,87]. Recent evidence of IRF-1 promoting the Th1 differentiation through the production of IL-12 by macrophages further supports the hypothesis that IRF-1 may link innate and adaptive immunity [88]. Interferon regulatory factor-1 is also involved in promoting an inflammatory response regulating the expression of inflammation-related enzymes, such as the inducible nitric oxide synthase and cyclooxygenase-2 and amplifying the TNF-induced vascular-cell adhesion molecule 1 expression [87,89]. These facts urged us to hypothesize that IRF-1 is involved in the pathogenesis of AIMs in myelodysplasia. In a study carried out in our department we examined the expression pattern of IRF-1 in 14 patients with MDS (seven without AIMs and seven with AIMs). In addition, five patients with systemic vasculitis and seven normal individuals were also studied and used as controls [90].

We found that MDS patients with AIMs displayed elevated, intact, full-length IRF-1 mRNA levels in their bone marrow mononuclear cells compared with MDS patients without AIMs. Recent findings implicate IRF-1 in vascular-cell adhesion molecule 1 expression (VCAM-1) and nitric oxide synthase production, adding a possible pathophysiological link between increased IRF-1 expression and vasculitic manifestations [87,91–93]. In our study, low expression of IRF-1 was established in all seven MDS patients without AIMs and this was in conjunction with exon skipping events, further supporting the possible role of IRF-1 gene inactivation in relation to the pathology of AIMs.

In this regard the absence of expression of IRF-1 appears to protect against the development of the vasculitic syndrome in MDS patients without AIMs. Our results are in agreement with in vivo models of IRF-1-deficient mice manifesting a reduced incidence and severity of type II collagen-induced arthritis and allergic encephalomyelitis [94]. These studies of IRF-1-deficient mice have revealed that mice lacking IRF-1 exhibited decreased incidence and severity of type II collagen-induced arthritis and experimental allergic encephalomyelitis compared with the wild-type mice. In these studies the authors not only implied the protective role of the absence of IRF-1 but also concluded that IRF-1 plays a role in promoting inflammation and autoimmunity. They also proposed that the inhibition of this transcription factor may be a novel therapy in controlling inflammatory diseases. Despite the fact that these experimental data cannot be applied to humans, however, our results point towards to the same direction.

The heterogeneity of clinical manifestations and prognosis among the different categories of MDS patients parallels...
their complexity regarding their pathogenetic events. In these complex genetic alterations the deregulation of the IRF-1 gene seems to be a critical oncogenic event in MDS patients without AIMs. However, the finding of a full-length IRF-1 mRNA expression in MDS patients with AIMs argues against the oncogenic role of IRF-1 inactivation in this specific group of patients, indicating another underlying mechanism of leukaemic transformation. In this regard we were unable to identify any particular difference in MDS patients with AIMs concerning their refractoriness to leukaemic evolution.

**Prognosis of myelodysplastic patients with autoimmune manifestations**

Previous retrospective studies have established a link between MDS and AIMs and illustrated interesting clinical aspects of this association. In these studies, it is highlighted that MDS/AIMS patients display poor prognosis [71,73, 74,95]. However, the prognostic significance of the coexisting autoimmune disorder in MDS could prove problematic owing to the fact that the underlying haematological disease is also remarkably heterogeneous in regard to the prognosis. These studies did not take into consideration the IPSS prognostic category and thus did not lead to firm conclusions regarding the exact clinical significance, the true prognosis and the survival of these MDS/AIMs patients. The IPSS, generated by multivariate analysis, is a prognostic model for MDS combining several prognostic features such as the percentage of marrow blasts, the degree of various cytopenias and the presence of chromosomal abnormalities [79]. The IPSS is a product of pooled data from previous scoring systems predicting survival and evolution to AML in MDS patients, and should therefore be considered when assessing the clinical significance and prognosis of AIMs in MDS.

In prospective, our study tried to determine the clinical aspects and evolution of AIMs in patients with MDS and to ascertain the prognostic implications of these manifestations in MDS [77]. All relevant information, including the IPSS prognostic category, cytogenetic analysis, treatment, evolution of the haemopathy, transformation to acute leukaemia and overall survival, was provided. It is worthy of note that the patients with AIMs were not statistically different in survival from the patients without AIMs; a finding that contrasts with previously published data [71,73,74,95]. No correlation between IPSS grade and predisposition to development of AIMs was observed. It should be emphasized that while the survival and prognosis of the MDS patients was not influenced by the presence of AIMs, IPSS was an important prognostic parameter in the MDS patients with AIMs. The fact that all the deaths in the group of MDS/AIMs patients were owing to myelodysplasia-related complications and occurred in the high-risk IPSS category further confirms our findings. We conclude that the prognosis and mortality of MDS patients manifesting overt AIMs is closely related to the haematological disorder.

**Conclusions**

Myelodysplastic syndrome with AIMs is an ideal model in the investigation of disordered immunological mechanisms in preleukaemic states. Systemic vasculitis, isolated autoimmune manifestations and connective tissue disorders are the main clinical manifestations in these patients. Despite the fact that several immunologic abnormalities have been described in human myelodysplasia, the precise pathophysiological mechanism underlying autoimmunity in MDS remains unclear. Although deletions as well as mutational and transcriptional inactivation of the IRF-1 gene have frequently been detected in MDS patients, those MDS patients with AIMs reveal an intact IRF-1 mRNA expression equal to that observed in patients with systemic vasculitis. In this regard the elevated expression of IRF-1 in a subset of MDS patients causes overactive interferon signalling, Th1 polarization and an abnormally overexpressed inflammatory process, all involved in the pathogenesis of vasculitic manifestations of these patients (Fig. 4). Further studies of IRF-1 expression and signalling will almost certainly reveal immunoregulatory changes in the preleukemic states and autoimmune systemic disorders.

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