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Evaluation of interleukin-18 levels in patients affected by multiple myeloma and monoclonal gammopathy of undetermined significance: analysis and review of the literature

A. ALLEGRA[1], D. VINCELLI[2], G. SPATARI[3], M. FERRARO[4], A. ALIBRANDI[5], G. MIRABILE[1], E. PACE[4], B. MARTINO[2], G. PIOGGIA[6], S. GANGEMI[7]

[1]Department of Human Pathology in Adulthood and Childhood "Gaetano Barresi", Division of Hematology, University of Messina, Messina, Italy
[2]Division of Hematology, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria, Italy
[3]Department of Biomedical and Dentistry Sciences and Morphological and Functional Imaging, University of Messina, Messina, Italy
[4]Institute of Translational Pharmacology (IFT), National Research Council of Italy (CNR), Palermo, Italy
[5]Department of Economics, Unit of Statistical and Mathematical Sciences, University of Messina, Messina, Italy
[6]Institute for Biomedical Research and Innovation (IRIB), National Research Council of Italy (CNR), Messina, Italy
[7]Department of Clinical and Experimental Medicine, Allergy and Clinical Immunology Unit, University of Messina, Messina, Italy

G. Pioggia and S. Gangemi have equally contributed to this work

ABSTRACT. – OBJECTIVE: Monoclonal gammopathy of undetermined significance (MGUS) is a preneoplastic disease that often precedes multiple myeloma. The multistep evolutionary pattern of multiple myeloma is driven by genetic instability, a pro-inflammatory and immunosuppressive microenvironment, and tumor growth. Inflammation has long been recognized as a factor in both the onset and progression of cancer.

PATIENTS AND METHODS: In this study, interleukin-18 plasma levels were compared in patients with multiple myeloma and monoclonal gammopathy of undetermined significance, as well as in a group of healthy controls.

RESULTS: Our study shows that monoclonal gammopathy of undetermined significance patients have lower levels of interleukin-18 than healthy controls (521.657 ± 168.493 pg/ml *vs.* 1,266.481 ± 658.091 pg/ml for controls, $p < 0.001$). Thus, we discovered a significant difference in interleukin-18 levels between multiple myeloma patients and controls (418.177 ± 197.837 pg/ml; $p = 0.001$).

CONCLUSIONS: In our work, we identified a reduction of interleukin-18 in monoclonal gammopathies. Furthermore, in this paper, we aimed to evaluate the existing literature on the potential mechanisms of action of this pro-inflammatory cytokine in the development of these diseases.

Key Words:
Multiple myeloma, Monoclonal gammopathy of undetermined significance, Interleukin-18, High mobility group box 1, Inflammation, Th1 immune response.

Introduction

Multiple myeloma (MM) is a plasma cell malignancy that develops in the bone marrow (BM) microenvironment as a result of aberrant plasma cell growth and proliferation. Plasma cells are supported in their development and are shielded from apoptosis by the secretion of different cytokines[1,2]. Three/four percent of the general population over the age of 50 have monoclonal gammopathy of undetermined significance (MGUS), which is the forerunner to malignant plasma cell disorders[3,4].

A desynchronized cytokine system with elevated levels of inflammatory cytokines and altered bone marrow milieu are typical features of MM[5-9], and various inflammatory factors may play a role in the development and progression of the disease.

Corresponding Author: Alessandro Allegra, MD; e-mail: aallegra@unime.it

The interleukin-1 (IL-1) family of cytokines and their associated receptors are primary signaling elements of inflammation[10]. As a physiologically inactive precursor, IL-18, a member of this superfamily of cytokines, is kept in the cytoplasm of the cells that produce it[11-14]. Nearly all human cells, including intestinal epithelial cells, keratinocytes, and endothelial cells, express IL-18 precursor[15,16].

Over the past twenty years, researchers have examined the pleiotropic effects of IL-18, and their understanding has become more complex. IL-18 mainly supports the Th-1 immune response by working with interleukin-12 to stimulate T, NK, and macrophage cells to produce IFN[17,18]. Furthermore, it has proinflammatory qualities because, as mentioned above, it triggers the inflammatory cytokine cascade. Furthermore, IL-18 is connected to the beginning of autoimmune diseases, and there is *in vitro* proof that IL-18 prevents osteoclast development[19,20].

However, in neoplastic diseases, other molecules appear to maintain the equilibrium between inflammatory and anti-inflammatory mediators. Numerous cancer types have shown evidence of high mobility group box 1 (HMGB-1) upregulation[21-26].

Extracellular HMGB-1 regulates the proliferation, maturation, and senescence of hematopoietic stem cells (HSCs). Through its stimulation of proinflammatory signaling pathways, HMGB-1 is also thought to play a role in the formation of the inflammatory BM micromilieu.

In a previous investigation[27], we assessed the plasma levels of psoriasin and HMGB-1 in individuals with monoclonal gammopathies (MM and MGUS) and the general population. The goal of the current investigation was to assess the plasma levels of IL-18 in the same cohort used in the prior study using the material stored at -80°C. The study's secondary goal was to assess the overall trend of the two molecules connected to inflammation in subjects with monoclonal gammopathies.

Patients and Methods

The study was carried out on three groups of subjects and was conducted in accordance with the ethical principles of the responsible committee on human experimentation and the Helsinki Declaration of 1975, as revised in 2013. The Local Ethics Committee approved this study protocol before the initiation of any study-related procedures (Protocol No. 36/18 of 07 May 2018 – resolution No. 887).

Blood samples from 14 newly diagnosed MM patients (7 males and 7 females; mean age 71 ± 12 years), 14 subjects with MGUS (6 males and 8 females; mean age 73 ± 13 years), and 19 healthy subjects (8 males and 11 females; mean age 69 ± 13 years) were obtained by venipuncture after each subject was informed about the research and signed an informed consent form.

According to the International Stage System[28], eight individuals had MM stage I, four had MM disease stage II, and two had stage III. Bone marrow plasmacytosis was 64% on average (range: 40-81%). Immunoglobulin G (IgG) and A (IgA) were the paraprotein classes in 10 cases and 4 patients, respectively. Four individuals had bone disease, and three patients had renal failure.

Regarding the MGUS group, immunoglobulin G (IgG) and A (IgA) paraprotein classes were present in 11 and 3 individuals, respectively.

Age requirements were greater than 18, IgG or IgA MGUS, or MM. IgM MGUS or MM, active infections, osteoporosis, pregnancy, scleroderma, use of glucocorticoids within three months, and cancer within five years were the exclusion criteria. Neither chemotherapy nor painkillers were being given to any patients.

Complete blood count with differential, platelet count, blood chemistry, beta-2 microglobulin, renal and liver function tests, calcemia, serum albumin, lactate dehydrogenase (LDH), immunoglobulin, erythrocyte sedimentation rate (ESR), and bone marrow aspiration were all part of routine laboratory examinations. Skeletal X-rays and physical examinations were conducted on all patients.

Immediately after being drawn, blood samples were centrifuged at 14,000 g for 20 minutes before being aliquoted into 1.5 ml centrifugation tubes. Until they were examined, samples were kept at -80°C.

Measurement of IL-18

Following the manufacturer's instructions, the manufacturer's enzyme-linked immunosorbent assay (ELISA) (Duo Set R&D Systems, Minneapolis, MN, USA) was used to measure the amount of IL-18 in the individuals' serum. The result was expressed in pg/mL.

Statistical Analysis

Due to the small sample size, which prevented confirmation of a Gaussian distribution of the

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data, the statistical analysis was conducted using non-parametrical tests. Therefore, a One-way Kruskal-Wallis ANOVA for independent samples was initially carried out to compare the three groups of persons (MGUS, MM, and controls).

Then, using pairwise comparisons and the post-hoc test, the appropriate comparisons were made, concentrating on the differences between the 2 groups. All statistical results were considered significant at a *p*-value threshold of 0.05.

Results

Our results show that IL-18 levels decreased over time from healthy individuals (1,266.481 ± 658.091 pg/mL) to patients with MGUS (521.657 ± 168.091) to those with MM (418.177 ± 197.837). One-way ANOVA revealed significant differences in IL-18 levels between the groups ($p < 0.001$) (Figure 1).

The post-hoc test with pairwise comparisons confirmed a significant difference between patients with monoclonal gammopathies and controls (Table I).

In our prior study[27], one-way ANOVA was used to detect changes in HMGB-1 levels between the groups ($p < 0.001$). The post-hoc test with pairwise comparisons made it possible to determine that there is a significant difference between patients with monoclonal gammopathies and controls, with a progressive increase in HMGB1 values from healthy controls to MGUS patients up to MM patients. There was a negative association found between HMGB-1 concentrations and creatinine clearance (r -0.667, $p < 0.049$), but no correlation was found between HMGB1 and the ISS stage, the presence of bone lesions, or the beta2m level in MM patients. There was no correlation seen between IL-18 levels and HMGB-1 concentrations.

Discussion

It has been well-established that inflammation is linked to the initiation and progression of cancer[28]. However, the literature about IL-18 concentrations in MM patients and their role in the onset and development of the disease appears to be contradictory[30,31].

Our data show that IL-18 values decreased over time as MGUS developed in healthy subjects and that a further decrease occurred in patients with MM, though it was not statistically significant. This information may appear to be at odds with some information found in the literature, particularly the findings of Alexandrakis et al[29]. In this work, authors revealed that blood IL-18 levels were raised in MM patients with advanced-stage disease; nevertheless, there was no significant difference in the overall survival between blood IL-18_{high} and IL-18_{low} MM patients[32].

However, while most of our patients were subjects in the early stages of the disease (8 patients

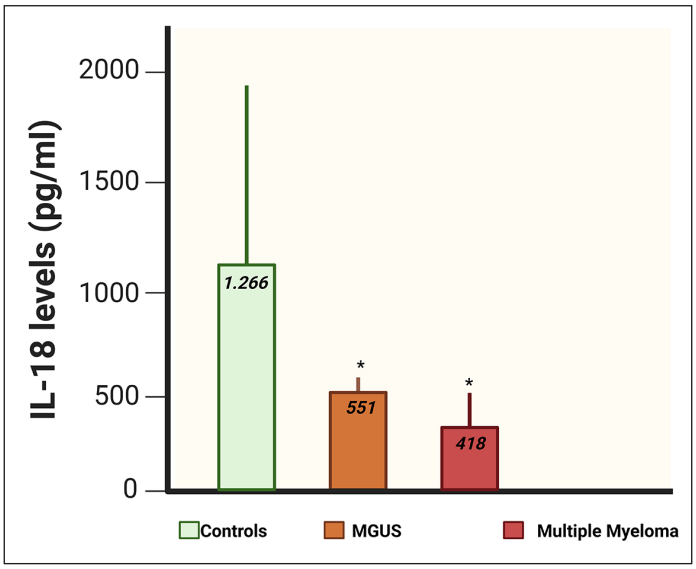

Figure 1. IL-18 levels in monoclonal gammopathies and controls; * $p < 0.001$ *vs*. controls. MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma.

were MM disease stage I, 4 patients were MM disease stage II, and only 2 patients were disease stage III) in this study, the increase in IL-18 values appeared to be present in the more advanced stages of the disease[32].

On the other hand, there is controversy over the real role IL-18 plays in the MM, and the data presented in the literature on its pro-tumor activity are conflicting[33].

Therefore, Nakamura et al[31] demonstrated that the MM niche's production of IL-18 promotes MM progression in a CD8+ T cell-dependent way in a mouse model, suggesting that IL-18 may be a potential therapeutic target in MM. In MM patients, high levels of BM IL-18 are linked to poor overall survival, while IL-18$^{-/-}$ mice were shown to be protected from MM progression in a CD8+ T cell-dependent way using Vk*MYC preclinical MM models. These findings suggested that IL-18 plays a critical role in the immunosuppression linked to MM by producing functional myeloid-derived suppressor cells (MDSCs). Comprehensive analysis of the immune microenvironment in MM patients' transcriptional landscape supported this theory by showing an inverse correlation between PMN-MDSC signature and cytotoxic signature and a positive correlation between IL18 and PMN-MDSC signature genes[31].

However, IL-18 has been proven to have an anticancer effect in other investigations. IL-18 and protein-10 co-injections utilizing adenoviral vectors led to tumor regression in murine myeloma cell lines[34]. Moreover, exogenous injection of high dose IL-18 caused a successful Th1 response in mouse plasmacytoma in a different research[35]. In syngeneic animals, a mouse myeloma cell line that has been IL-18 transfected showed reduced tumorigenicity[36]. Finally, in a fascinating study, IL-18 treatment reduced the infiltration and development of human myeloma cells into the bone marrow in mice with severe combined immunodeficiency illness, suggesting that IL-18 may have anticancer effects in MM[37].

Table I. Significantly different IL-18 levels between monoclonal gammopathies patients and controls.

Terms of the statistical comparison	*p*
MGUS *vs.* MM	0.810
MGUS *vs.* Controls	0.0001
MM *vs.* MGUS	0.810
MM *vs.* Controls	0.0001
Controls *vs.* MGUS	0.0001
Controls *vs.* MM	0.0001

MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma.

The dispute around the data may be addressed by the possibility that IL-18 injected exogenously may cause a different reaction than IL-18 produced naturally and that the quantities of the cytokine in both situations are not comparable. It has been hypothesized that IL-18 may predominantly stimulate NK cells and produce tumor-specific cytotoxic CD4+ T cells to exhibit its antitumor function[38].

Furthermore, there is evidence to suggest that IL-18's anti-angiogenic activity can account for its anticancer effects[39]. It is widely acknowledged that angiogenesis plays a crucial role in the development of malignant tumors[40,41], and the pathophysiology of MM depends on the creation of new arteries from pre-existing vasculature[42]. Numerous plasma and stromal cell-derived factors have been linked to the progression of angiogenesis in MM, even if the precise molecular processes governing this phenomenon are still unknown[43].

However, there is also conflicting information regarding the function of IL-18 in angiogenesis in this condition. *In vivo* in mice, IL-18 reduces the proliferation of capillary endothelial cells induced by fibroblast growth factor-2[39,44,45]. However, IL-18 has been classified as an angiogenic molecule in another research. In both *in vitro* and *in vivo* mouse model experiments, IL-18 was proven to be a powerful angiogenic molecule. According to a previous study[46], IL-18 and IL-1 increased liver metastases and melanoma cell adhesion by boosting the expression of vascular cell adhesion molecules on hepatic sinusoidal endothelium. Moreover, in systemic lupus erythematosus, IL-18 has been investigated and found to be elevated in comparison to controls but positively linked with endostatin, a protein thought to promote angiogenesis[47].

Considering the above, the variation of IL-18 levels in the spectrum of gammopathies can be interpreted as a reduction of a cytokine with antitumor activity, and this reduction could play a role in the onset and progression of myeloma disease.

In terms of HMGB-1, we previously demonstrated that patients with MGUS had larger quantities than healthy controls[27]. Moreover, we discovered a significant variation in HMGB-1 levels between MM patients and controls, and our data seemed to corroborate with Wang et al's findings[48]. In this investigation, the blood HMGB-1 levels of the patients in the MM group were greater than those in the control group prior to therapy.

In addition, patients in the Complete Response group had HMGB-1 levels that were lower post-chemotherapy compared to pre-chemotherapy levels, while those in the Progressive Disease group had increased HMGB-1 levels[48]. Additionally, it is recognized that the molecule might be crucial to the development of cancer by interacting with receptors for advanced glycosylation end-products (RAGE)[49]. The HMGB-1 that tumor cells produce may worsen the immunosuppression brought on by inflammation in malignancies that have already spread. Additionally, HMGB-1 enhanced the epithelial-mesenchymal transition by promoting angiogenesis and the formation of tumor-like features in neoplastic cells. The NF-kB pathway is stimulated by the increased HMGB-1/RAGE complex, which results in the synthesis of proangiogenic substances such as vascular endothelial growth factor (VEGF) and their receptors. VEGF and its receptors play an important role in the development of hematological malignancies[50]. Furthermore, HMGB-1 can change several pathways involved in programmed cell death[51].

Our findings appear to support the notion that MM patients have higher HMGB-1 levels and lower IL-18 concentrations than patients with benign gammopathy and healthy individuals. It may seem unusual that two drugs that are largely pro-inflammatory should behave differently within the same condition. The link between inflammatory cytokines and MM, however, seems to be weak, and other roles, like pro- or anti-proliferative effects, can step in for cytokines in the development and progression of MM. When regulated by Th1 cells that are specifically anti-cancer, inflammation may prevent the onset and growth of tumors. Proinflammatory cytokines (including IL-6 and IL-1) may aid in the elimination of tumors in a Th1 microenvironment by luring leucocytes from the bloodstream and boosting CD4 + T cell activity[7]. Consequently, care should be taken when thinking about therapies that focus on substances having pro- or anti-inflammatory activity. Medication that may reduce the Th1-driven inflammatory immune response—which inhibits the growth of tumors—should be avoided.

Additionally, as mentioned, IL-18 fundamentally supports the Th-1 immune response by collaborating with interleukin-12 to promote the production of IFN by T cells, NK, and macrophages. However, IL-18 is a special cytokine that may activate both Th1 and Th2 responses depending on its cytokine milieu, and this feature may also help to explain some of the contradictory information about the cytokine's effects that occasionally appear in the literature[52].

It should be remembered that the levels of the factors in the marrow microenvironment may differ from those in the peripheral blood and that various disease stages may impact the generation of the factors. Furthermore, ethical issues have made it impossible to compare the medullary concentrations of the factors with either a healthy subject or with MGUS.

Despite this limitation, along with the small sample size examined, our initial data seem to align with some findings reported in the literature. It will be feasible to clearly characterize the pro- or antitumor role of IL-18 through further research and clarify the differences between the data that are now available in the literature. This will make it possible to put therapy plans in place that attempt to change the cytokine's concentrations and activity. Therefore, by increasing the number of cell populations with anti-myeloma activity, *in vitro* studies have already shown the value of IL-18 in the treatment of MM[37].

Conclusions

Although NK cell-based immunotherapy is a promising treatment option for MM, it is still difficult to obtain enough activated NK cells. According to a previous study[30], K562 cells were genetically modified to express OX40 ligand, membrane-bound (mb) IL-18, and membrane-bound (mb) IL-21 to create *ex vivo* expanded NK (eNK) cells from MM patients. Moreover, eNK cells demonstrated higher expression of key activation markers and greater cytotoxicity towards target K562, U266, and RPMI8226 cells[30].

The control of cytokines and the modification of the BM microenvironment may one day play a key role in the management of multiple myeloma.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

The study was carried out on three groups of subjects and was conducted in accordance with the ethical principles of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. The Local Ethics Committee of Polyclinic "G. Martino" of Messina (Italy) approved this study protocol before the initiation of any study-related procedures (Protocol No. 36/18 of 07 May 2018 – resolution No. 887).

Informed Consent
All patients signed an informed consent form.

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Authors' Contributions
Alessandro Allegra, Donatella Vincelli, Bruno Martino, Giovanna Spatari, Giuseppe Mirabile, Giovanni Pioggia, Sebastiano Gangemi: supervision and editing; Maria Ferraro, Elisabetta Pace: formal analysis; Angela Alibrandi; Alessandro Allegra: data analysis; Alessandro Allegra: writing, original draft; Alessandro Allegra, Donatella Vincelli, Bruno Martino, Giovanna Spatari, Giuseppe Mirabile, Giovanni Pioggia, Sebastiano Gangemi: editing and review. All authors have read and agreed to the published version of the manuscript.

ORCID ID
Alessandro Allegra: 0000-0001-6156-8239
Giovanna Spatari: 0000-0002-6716-7572
Maria Ferraro: 0000-0003-2533-7446
Elisabetta Pace: 0000-0003-1141-9010
Giovanni Pioggia: 0000-0002-8089-7449
Sebastiano Gangemi: 0000-0001-7001-6532

Data Availability
Data are available upon request from the authors.

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