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Short Communication

Altered cytokine and chemokine profiles in multiple myeloma and its precursor disease

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ABSTRACT

Currently, no reliable biomarkers are available to predict transformation from smoldering myeloma (SMM) to multiple myeloma (MM). Using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) we assessed the levels of a broad range of cytokines and chemokines in the peripheral blood (PB) and bone marrow (BM) supernatant collected from 14 SMM and 38 MM patients and compared to healthy donors. We found significantly increased levels of key cytokines, in particular CXCL8 (IL-8), associated with progressive disease state (controls → SMM → MM). Cytokine profiles were found similar in PB and BM. Five of fourteen SMM patients (36%) progressed to MM.

Our findings, although based on a limited number of patients, suggest that serum-based cytokines may have a future role as biomarkers for disease progression and could potentially be assessed as novel targets for treatment.

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1. Introduction

Currently, two clinical risk models (Mayo Clinic and Spanish PETHEMA model) are available to predict progression from smoldering myeloma (SMM) to multiple myeloma (MM) [1,2]. Both these models are based on data derived from retrospective single-center studies. Recently, a prospective head-to-head comparison of the two models showed a high degree of discordance when defining individual patients risk using the two models in parallel [3].

In order to expand our knowledge on biological markers of progression, we assessed a broad range of cytokines and chemokines proposed to play a role in myelomagenesis [4], including IL-1β, IL-2, IL-4, IL-5, IL-6, CXCL8, IL-10, IL-12 p70, IL-13, IFNγ, and TNFα. We assayed these markers in peripheral blood and bone marrow supernatant from SMM and MM patients and compared our results to samples obtained from healthy donor controls.

2. Patients and methods

Bone marrow and peripheral blood samples (serum) were obtained with informed patient consent. Peripheral blood samples were obtained from 7 healthy donors, 14 SMM patients and 38 MM patients. BM supernatant samples were obtained from 17 MM patients and from 9 healthy donors. The samples were assayed in two ELISA multi-array experiments using an ultra-sensitive Human TH1/TH2 10-plex multi-spot plate and an ultra-sensitive multi-array plate for IL-6 detection (Meso Scale Discovery®). A set of known concentration calibrators was added to the 96-well plate to generate a standard curve for each cytokine. The derived standard curves were used to calculate cytokine concentrations in each of the clinical samples. All samples were added to the plates in duplicate. The assays were repeated twice (Fig. 1). A two-tailed Mann–Whitney test was performed for statistical analysis using Prism software. Gene expression profile (GEP) data were collected from the Gene Expression Omnibus accession number GSE6477. All microarray data derived from Affymetrix U133A chip.

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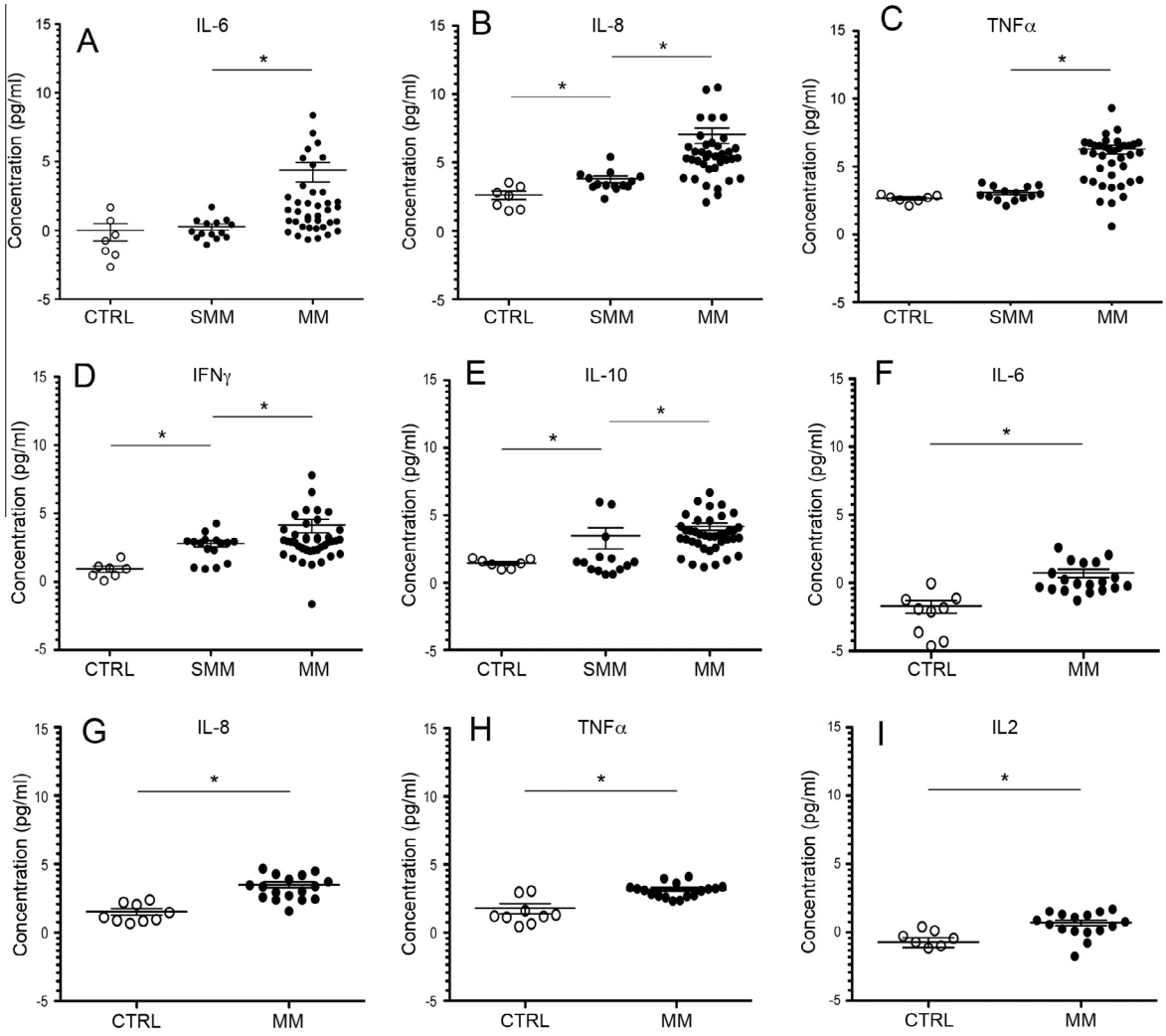

Fig. 1. Comparison of cytokine and chemokine levels in peripheral blood (PB) and bone marrow (BM) supernatant of SMM and MM patients. PB (serum). (A) Values are expressed as log2. Level of IL-6 significantly elevated in MM patients (21 ± 9.5 pg/mL) compared to SMM (1.2 ± 0.7 pg/mL) compared to controls (1.0 ± 0.4 pg/mL) $^{*}p = 0.006$ and $^{*}p = 0.001$ respectively. (B) CXCL8 (or IL-8) concentration elevated in SMM patients (13.9 ± 2.4 pg/mL) and MM patients (133.2 ± 48 pg/mL) versus controls (6.2 ± 1.2 pg/mL $^{*}p = 0.008$ and $^{*}p < 0.0001$ respectively. (C) TNF-alpha level increased in SMM patients (8.5 ± 2.9 pg/mL) and MM (78 ± 16.9 pg/mL) compared to controls (6.3 ± 1.1 pg/mL). MM versus SMM: $^{*}p = 0.0005$). (D) Levels of IFN-gamma in SMM (7.0 ± 1.1 pg/mL) and in MM (17.9 ± 5.9 pg/mL) compared to controls (1.9 ± 0.3 pg/ml) $^{*}p = 0.002$ and 0.001 respectively. (E) Level of IL-10 significantly increased in MM patients (18.08 pg/mL ± 3.2) versus SMM (11.16 ± 5.4) and controls (2.7 ± 0.6 pg/mL) $^{*}p = 0.001$ and 0.0003 respectively. BM supernatant. (F) Level of IL-6 increased in MM patients (1.66 ± 0.3 pg/mL) compared to controls (0.3 ± 0.09 pg/ml) $^{*}p = 0.0003$. (G) Level of CXCL8 increased in MM patients (11.3 ± 1.6 pg/mL) compared to controls (2.8 ± 0.4 pg/mL) $^{*}p = 0.0001$. (H) TNF-alpha elevated in MM patients (9.03 ± 0.8 pg/mL) compared to controls (3.49 ± 0.8 pg/mL) $^{*}p = 0.0008$. (I) Level of IL-2 elevated in MM patients (1.6 ± 0.2 pg/mL) versus controls (0.6 ± 0.1 pg/mL) $^{*}p = 0.007$. All samples were assayed in two ELISA multi-array experiments using an ultra-sensitive Human TH1/TH2 10-plex multi-spot plate for quantifying the levels of IL-1β, IL-2, IL-4, IL-5, CXCL-8, IL-10, IL-12 p70, IL-13, IFN-gamma, TNF-alpha; and an ultra-sensitive multi-array plate for IL-6 detection (Meso Scale Discovery®).

3. Results and discussion

Using a multi-array assay, we quantified the level of a broad range of cytokines and chemokines both in PB blood and bone marrow supernatants of SMM, MM patients and healthy donors. In PB obtained from SMM patients, we found significantly increased levels of CXCL8 (IL-8) ($p = 0.008$) and IFNγ ($p = 0.002$) compared to healthy controls (Fig. 1). The same cytokines were found to be further increased in PB from MM patients compared to SMM and controls: CXCL8 ($p = 0.0009$ and $p \leqslant 0.0001$); IFNγ ($p = 0.001$ MM versus controls) (Fig. 1). Furthermore, additional cytokines were elevated in PB of MM patients compared to SMM and controls including: IL-6 ($p = 0.0009$, $p = 0.003$) and IL-10 ($p = 0.001$, $p = 0.0003$); TNF-alpha was elevated in MM versus controls ($p = 00005$). In addition, in the PB of three of SMM patients we also found elevated concentrations of IL-10 compared to controls (Fig. 1).

As a second step, we assessed patterns of cytokines assayed in BM supernatants derived from 38 MM tumors and found a profile similar to that defined in PB. Specifically, levels of IL-6, CXCL8 and TNFα were significantly greatly increased in MM patients compared to controls ($p = 0.0003$, $p = 0.0001$, $p = 0.0008$,). Levels of IL-2 were also increased in BM of MM patients ($p = 0.007$) (Fig. 1). Our findings are consistent with a previous study showing high levels of chemokine IL-8 in SMM and MM patients in an *in vitro* model of human stromal cells cultured in the presence of BM supernatant derived from MGUS, SMM and MM patients [5].

Of 14 SMM patients analyzed, five (36%) progressed to MM in a two-year follow-up (Fig. 2A). Although the statistical difference in the level of CXCL8 between the group of SMM patients with progressive disease (PD) and the rest of SMM patients with stable disease (SD) was limited due to sample size, the mean concentration of CXCL8 in PD patients was elevated threefold