Overproduction of IL-7, IL-10 and TGF- β 1 in Multiple Myeloma

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Multiple myeloma (MM) is characterized by bone marrow infiltration of myeloma cells, a monoclonal gammopathy, and osteolytic lesions. Although most patients respond to initial chemotherapy, nearly all patients relapse and are refractory to salvage regimens. It is reasonable to assume that the outcome is related to a multidrug-resistant subset of circulating myeloma cells [1]. The bone marrow infiltration by this particular cell population and the growth of myeloma cells inside osteolytic lesions are regulated by a cytokine network where interleukin-6 (IL-6) plays a key role.

High serum levels of IL-6 or IL-6 receptor (IL-6R) are identified as risk factors in MM. IL-6 is suggested to act as autocrine or paracrine growth factor for myeloma cells produced by the tumour environment [2]. Additionally, B cell-regulating cytokines such as IL-7, IL-10 and IL-13 are proposed to be involved in myeloma development [3]. Furthermore, IL-4 is able to sustain the viability of activated B cells in vitro but together with TGF- β 1 it inhibits IL-6 synthesis [4]. In a recent report it was shown that high serum level of IL-2 are associated with a smouldering course of MM. In addition, IL-7 and IL-13 have been shown to increase the level of IL-2-induced activity of lymphokine-activated killer cells supporting immunological defence mechanisms against B cell malignancies, but IL-4 inhibits these effects [5, 6].

The object of this investigation was to evaluate in vivo serum levels of IL-2, IL-4, IL-6, IL-6R, IL-7, IL-10, IL-13

and TGF- β 1 in different stages of MM. With respect to the hypothesis that cytokines have more localised effects, analyses of peripheral blood and bone marrow aspirates were performed in parallel. Using the nonparametric Wilcoxon-Mann-Whitney U test for statistical analysis these parameters were correlated with clinical features and different B and T cell antigens on

lymphocytes to determine a predictive value. The cytokines were assayed in plasma probes of peripheral blood and bone marrow aspirates using solid-phase sandwich ELISA (Biermann, Germany). Immunophenotyping of mononuclear cells after density gradient centrifugation (Ficoll-Hypaque) was studied by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) using monoclonal antibodies [CD3-FITC(UCHL1), CD4-FITC(13B8.2), CD16-FITC(3G8), CD14-FITC(RM052) and CD38-FITC(T16) all from Coulter-Immunotech, Hamburg, Germany].

According to the Durie and Salmon classification of MM 23 patients were investigated: 6 patients in stage I, 11 patients in stage II and 6 patients in stage III. The clinical features are shown in table 1. Serum β 2-microglobulin (β -2M) levels were identified as the best parameter correlating significantly with tumour burden, serum M component ($p < 0.05$), serum haemoglobin ($p < 0.04$) and C-reactive protein ($p < 0.02$). To evaluate a clinical relevance, the investigated cytokine levels were correlated with a standard reference interval and β -2M.

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Table 1. Clinical characteristics of 23 tested MM patients

Parameters

Values

Age, years

Mean

63

Range

33-82

Sex (m/f)

8/15

Stage

I

6

II

11

III

6

Type

IgG

20

IgA

2

IgM

f

Skeletal

0

8

destruction**b**

1

5

2

10

Serum **M**

< **11** g/l

7

component**0**

≥ **11** g/l

16

CrPd

< 8 mg/l

14

> 8 mg/l

9

B-2Me

< 2.4 mg/l

18

> 2.4 mg/l

5

CrP = C-reactive protein. **a** From Durie and Salmon [9]. **b** Number of osteolytic lesions: 0 = normal bone structure, 1 = solitary bone lesion, 2 = multiple bone lesions. **c** Reference interval: 7.26-**17** g/l. **d** Reference interval: 0.068-8.2 mg/l. **e** Reference interval: 0.8-2.4 mg/l. **f** The diagnosis was confirmed by the following results: plasma cell infiltration of the bone marrow (20%) expressing cytoplasmic IgM using APAAP staining, multiple osteolytic lesions and a monoclonal M component of the serum without adenopathy or splenomegaly.

The results are shown in table 2. No differences of cytokine concentrations were found in peripheral blood and bone marrow aspirates. With respect to osteolytic lesions, bone marrow aspirate analysis did not result in superior information. As expected, significantly higher IL-6 serum levels were measured in comparison to the reference interval ($p < 0.006$) and a significant correlation was found to increased β -2M in peripheral blood ($p < 0.0001$) as well as in bone marrow aspirates ($p < 0.0003$). In contrast, the IL-6R concentration was not increased. Similarly, IL-4 known as an IL-6 antagonist and IL-13 were not detectable.

The flow-cytometric analysis of mononuclear cells reveals an increase in monocytes (CD45+/CD**D14**++) in pe-

Table 2. Cytokine serum concentration and surface phenotype of mononuclear cells in peripheral blood (PB) and blood from bone marrow aspirates (BM) from MM patients divided into a group with low tumour burden (β -2M < 2.4 mg/l) and a group with high tumour burden (β 2-M > 2.4 mg/l)

IL-2R, U/ml
PB 874 ± 308 788 ± 205 1,079 ± 331 1,736 ± 435

BM 820 ± 259 764 ± 174 1,113 ± 264

0.3 ± 0.7 0.0 ± 0.0

80 ± 42

8.2 ± 6.5

< 0.1 3.5 ± 1.6

0 ± 0

0 ± 0

0 ± 0

IL-4, pg/ml PB + BM

+ 3.3 0 ± 0 4.1 ± 4.4

+ 4.3 0.05 ± 0.16 4.2 ± 6.9

IL-6, pg/ml PB BM

102 ± 93 73 ± 27

72 ± 23 67 ± 17

24 ± 27 28 ± 23

32 ± 21 37 ± 22

138 ± 149

86 ± 25

22 ± 27 29 ± 23

0 ± 0

IL-6R, ng/ml PB

BM

IL-7, pg/ml PB BM

IL-13, pg/ml PB + BM

0 ± 0

0 ± 0

Latent TGF- β , ng/ml

PB 9.5 ± 7.4 6.9 ± 2.1 12.9 ± 11.1

BM 10.8 ± 3.5 11.7 ± 3.2 9.4 ± 3.6

CD3, %

PB

26 ± 25

28 ± 29

18 ± 21

60 ± 8
BM
12 + 6
13 + 7
12 + 7
10 + 7
CD4, %

PB
20+15
19 + 18
23+11
38 + 7
BM
18+11
14 + 7
23 + 15
5 ± 3
CD19,%

PB
6 ± 5
7 ± 6
5 ± 3
11 + 5
BM
4 ± 4
4 ± 5
2 ± 2
4 ± 2
CD16,%

PB
17 + 7
12 + 6
20 ± 8
13 + 5

BM
10 + 5
10 + 5
10 ± 7
12 ± 6
CD14, %

PB
12 + 9
8 ± 6
20 ± 10
13 + 5
BM
4 ± 3
3 ± 2
6 ± 5
4 ± 3
CD38, %

PB
14 + 9
11 + 6
22+13

BM
23+ 15
21 + 11
24 ± 22
6 ± 5

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ipheral blood ($p < 0.015$). It remains speculative whether this phenomenon is associated with increased cytokine levels, especially for IL-6. In addition, a reduction of CD3+ cells (T cells) was found with a decrease of CD4+ / CD3+ T cells reflecting an immune deficiency in MM. However, IL-2 serum levels were significantly decreased in stage I and stage II disease ($p < 0.022$) and not detectable in stage III disease. Slightly enhanced IL-2 concentrations in patients with low tumour burden (β -2M > 2.4 g/l) could reflect a possible T cell-mediated antitumour effector role.

Significantly enhanced cytokine levels in comparison to the reference interval were found for IL-7, IL-10 and TGF- β ($p < 0.001$). IL-10 is assumed to be secreted by the myeloma cells, but the source of increased cytokine levels for IL-7 and TGF- β is not clear. Recently, it was shown that human myeloma cell lines are able to produce autocrine IL-10 and induce IL-11 receptor gene expression. The authors assume that IL-10 acts as an IL-6-unrelated autocrine growth factor for malignant plasmablastic cells and induces proliferation but not differentiation in human myeloma cells [7]. According to earlier studies TGF- β mRNA is weakly expressed in human myeloma cells and the growth of human myeloma cells is inhibited by TGF- β [8]. IL-7, known as bone marrow stromal-derived cytokine, is involved in B cell maturation and possibly also in myeloma development. The results presented strongly suggest that IL-7 and TGF- β may be involved in myeloma growth, but the specific biological functions are still unknown.

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