Eur J Cancer Clin Oncol, Vol. 24, No. 8, pp. 1295–1298, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

Alteration of Beta-2-microglobulin Level in Malignant Lymphoproliferative Diseases after a High Dose of Alpha-2-recombinant Interferon

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Abstract—*A group of 10 patients with malignant lymphoproliferative diseases resistant to any standard therapeutical modalities were treated with a high dose of alpha-2-recombinant interferon (alpha-2-rIF). Alpha-2-rIF was administered at a total dose of* 120×10^6 *IU in a continuous infusion during* 48 h. *Two cycles of alpha-2-rIF immunotherapy were employed with an interval of* 1 *month in between each. Serum and urinary beta-2-microglobulin (B2M) were examined prior to alpha-2-rIF application and on days* 2, 4, 6 *afterwards. Alpha-2-rIF treatment induced an increase in serum B2M as noted on day* 2 *followed by a decline to below the normal range. The initial increased value was significantly higher as compared to either the pretreatment value or the normal physiological range. The reduced B2M serum level was protracted and lasted until the second cycle of treatment. Similar but not so great changes in B2M serum values were noted during the second application of alpha-2-rIF. The changes of B2M level in the urine, although less convincing, mimic those observed in the serum. The present results confirmed the biological activity of alpha-2-rIF in malignant lymphoproliferative diseases.*

INTRODUCTION

BETA-2-MICROGLOBULIN (B2M) is a low molecular weight protein (11.8 kd) discovered in 1968 [1]. It is found in the body in two forms, i.e. as a free molecule and/or non-covalently bound to HLA antigens. Serum B2M represents its free form [2]. It has been observed that tumour cells in culture synthesize significantly higher amounts of B2M compared to normal cells. Some authors consider B2M as a possible marker of malignant diseases [3, 4].

Many studies, mostly performed *in vitro*, have shown that interferon (IF) increases the expression of various antigens including HLA and B2M [5]. An increase in serum B2M in relation to the dose of gamma-IF was recently observed [6, 7].

Cooper *et al.* [8] reported a marked increase of B2M serum level in patients with malignant lymphoproliferative diseases and chronic lymphocytic leukaemia. Similar results were described in patients with non-Hodgkin's lymphoma [9], in advanced stages of malignant lymphomas and in acute leukaemias with leukocytosis [10]. The correlation between B2M serum level and the stage of malignant lymphoma was further confirmed by other authors [11, 12]. These investigators found a significant increase of serum B2M in advanced stages of disease and they claim that B2M serum level has no fundamental prognostic significance. In addition, these authors [12] have described the significant decrease of serum B2M in patients who respond to chemotherapy.

In our study we have looked for a correlation between serum and urinary B2M levels and response to therapy in patients with malignant lymphoproliferative diseases treated with alpha-2-recombinant interferon (alpha-2-rIF).

MATERIALS AND METHODS

The group of 10 patients (5 male, 5 female) with malignant lymphoproliferative diseases consisted of two patients with acute lymphoblastic leukaemia, three patients with malignant lymphogranuloma (mixed cellularity 1, nodular sclerosis 2) and five patients with non-Hodgkin's lymphoma (centrocytic–centroblastic 1, centroblastic 3, lymphoblastic 1). The average age was 43 years. All patients suffered from persistent disease which has

Accepted 9 March 1988.
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not responded to any therapeutic modality. All malignant lymphoma patients had stage IV disease at the time of IF application as verified by X-ray or biopsy findings.

Alpha-2-rIF (Boehringer, Ingelheim, F.R.G.) was administered by i.v. drip at a total dose of 120×10^6 IU during 48 h (the content of a commercial vial is 5×10^6 IU). The interferon was dissolved in normal saline and fresh solution was prepared every 2 h. The interferon treatment was repeated at a 1 month interval.

B2M level was measured in serum and urine by means of standard RIA method using B2M monoclonal antibody labeled with ^{125}I (RIA kit VVVR, Prague, Czechoslovakia). The following are the normal levels according to this method: serum 2400–3000 μg/l, urine < 300 μg/l. Four samples of serum and urine were collected from each patient according to the following schedule: prior to treatment with IF then on days 2, 4, 6 after initiation of the IF infusion. The IF treatment and laboratory examinations were repeated after 1 month. The samples of serum and urine were kept at a temperature of −20°C until further procedures.

Apart from all standard biochemical and haematological examinations, the serum creatinine level was of a special interest.

Student's *t* test was used to evaluate the statistical significance of the results.

RESULTS

The changes in serum B2M in patients who received IF treatment are shown in Fig. 1. The mean of the initial B2M values was within normal limits. The mean value of B2M taken 48 h after IF infusion was significantly higher (P 0.02) than the initial value. There was a profound fall in serum B2M in both successive measurements, i.e. on day 4 and day 6, respectively, the latter value has dropped even below the normal range. Similar

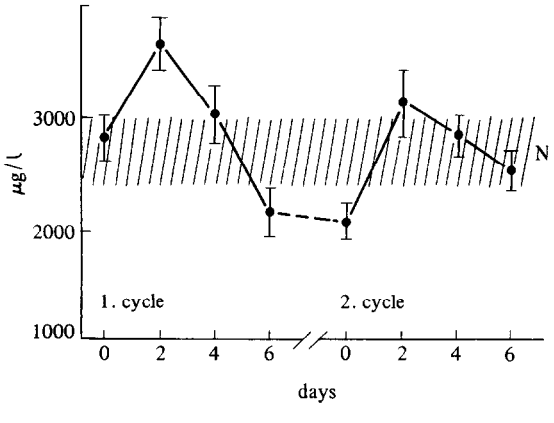

Fig. 1. The changes of serum B2M in 10 patients with lymphoproliferative malignancies during the course of alpha-2-rIF therapy. Alpha-2-rIF was administered via an i.v. drip in a single dose of 120 × 10⁶ IU in two cycles with a 1 month interval between each.

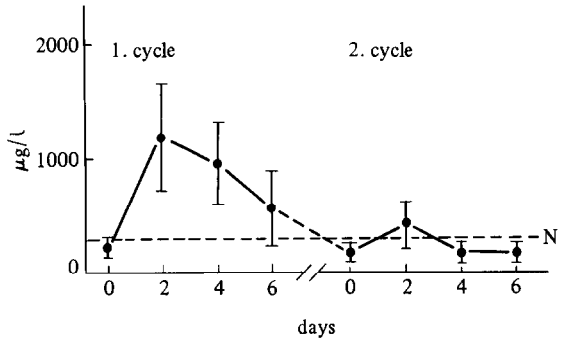

Fig. 2. The course of changes of B2M in the urine in selected patients with lymphoproliferative malignancies during the alpha-2-rIF treatment. For the IF dosage see legend to Fig. 1.

patterns of B2M serum level changes (increase followed by decline) were observed in all of the 10 patients examined. As for the value measured on day 6 it is significantly lower when compared with the initial one (P 0.05) and, simultaneously, significantly lower in comparison with B2M level determined immediately after IF infusion (P 0.01).

The interval between the first and second treatments with IF was 1 month. The initial B2M level in the second cycle of IF treatment was below the normal range. This value was even significantly lower than the initial one (P 0.02). After IF the serum B2M level was significantly higher than the pretreatment value (P 0.01). The increase noted 48 h after giving IF is followed by another drop into the normal range and the difference between values (day 2 vs. day 6) is apparent but not significant (P 0.10).

The results of urinary B2M excretion (see Fig. 2) were affected by the low number of samples (menstruation in some patients). Due to the inadequate number of samples (< 10) no statistical evaluation was made. Nevertheless, the B2M level in urine corresponds with the changes in serum B2M level. It is evident from Fig. 2 that the B2M changes in urine are not as illustrative as in the serum. The measured excretion of B2M in the urine lasts rather longer time after treatment with IF.

DISCUSSION

It is assumed that IF exerts a wide variety of biological activities, of which the antitumour effect is mainly mediated by its direct cytostatic, antiproliferative and immunological mechanisms [13, 14]. In *in vitro* experiments it has been shown that IF increases the expression of B2M and HLA antigens by lymphocytes [5]. As proved before, mainly the higher expression of Class II antigens is concerned (HLA-DR) where all three types of IF exhibit similar activity [15]. In our case, the increased serum B2M level observed after alpha-2-rIF administration to patients with malignant lymphoproliferative diseases gives no possibility of deciding if B2M

has been preferentially secreted from tumour or normal cells. Recent studies [6, 7] prove that serum B2M increases in patients with various malignant diseases, including malignant lymphomas. After gamma-IF treatment, an elevated serum B2M level occurred after 7 days from IF infusion. Of course we should contemplate a protracted effect of gamma-IF in comparison with alpha-2-rIF employed in our study, which means that a further increase of B2M could be caused by prolonged stimulation of lymphocytes. Similarly, our data indicate a significant serum B2M increase immediately after the cessation of the alpha-2-rIF infusion but, in contrast to previous reports, there was a significant decrease in serum B2M level on the 6th day. This fall in serum B2M corresponds to the decreased serum B2M in malignant lymphomas after effective chemotherapy [12].

The interesting findings in the B2M changes were obtained during the second cycle of alpha-2-rIF treatment, i.e. 1 month after the first application. The B2M serum level remains decreased for the whole period in between the two applications of alpha-2-rIF as demonstrated by the initial value of serum B2M taken prior to the second alpha-2-rIF cycle. Again, this serum B2M level is within the normal range. Alpha-2-rIF caused an increase in serum B2M on the 2nd day followed by a decline to the normal range. It is supposed that the differences between the B2M level changes caused by successive alpha-2-rIF applications might be due to as yet unknown immune mechanisms, which are less responsive to the second exposure to alpha-2-rIF. Alternatively, the capacity of tumour cell membranes to express B2M has been somewhat altered so that the response to repeated applications of the alpha-2-rIF with respect to B2M secretion is lower. In addition, a reduced target mass may also be involved. Furthermore, the combination of each above mentioned mechanisms should also be considered.

Measurement of B2M in the urine does not contribute significantly to the clarification of changes induced by the interferon. It appears that the urinary B2M level corresponds to a certain degree to the serum B2M level with the only difference being a more protracted duration of the increased urinary level. It is not clear so far whether the direct effect of IF on the kidney tubules induces the changes of urinary B2M but this mechanism cannot be excluded. Since a rise in serum B2M level have been described in connection with kidney diseases [16], we have also monitored the serum creatinine level to reveal any pathological kidney function as being associated with our findings. We did not find any changes in the creatinine serum level either in the initial stage or during the post alpha-2-rIF application period when the toxic effect on renal function could be considered [17].

The stimulation of lymphocytes mentioned as a main cause of the increase in B2M level was reported in numerous diseases such as haematological malignancies [8–12], virus infections (especially by cytomegalovirus), infection mononucleosis and influenza type A [18], AIDS and suspected AIDS [19] and in patients with kidney graft during rejection episodes [20]. A decrease in B2M serum level has been noted in malignant lymphomas after effective chemotherapy and has been related to cytostatic effect [12]. The present study indicates that the course of B2M serum level changes, i.e. a rise followed by a decrease, may be brought about by alpha-2-rIF.

In conclusion, the changes in serum B2M level reported in this paper were induced by a high dose of alpha-2-rIF which confirms the significant biological and clinical activity of this biological response modifier. As stated before it appears that the changes in B2M after IF application reflect its well recognized immunostimulation combined with direct cytostatic activity. However, the significance of B2M determination in relation to response to IF therapy and/or deciding therapeutic strategy remains to be established.

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