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Involvement of β2-Microglobulin in CD69 Expression on T Cells

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Abstract. β_2-Microglobulin (β_2M) is the light chain of the class I HLA molecule. The serum level of β_2M is elevated in various diseases including lymphoma, inflammation, viral infections and chronic renal dysfunction. The present study addressed the possible influence of β_2M on T lymphocyte activation *in vitro*. Peripheral blood mononuclear cells from a group of 17 healthy subjects were examined. Stimulation with OKT3 and fibronectin in combination with 30 mg β_2M/dl resulted in a two-fold increase of cell proliferation. A similar effect was observed when OKT3 and collagen I were applied as well as when OKT3 and collagen IV were used as costimulation to T cells. The CD69 expression, measured by flow cytometry was significantly enhanced above the control level (1.52 ± 1.03% vs 33.21 ± 20.26%, $p<0.01$, control group and 30 mg β_2M/dl, respectively). Together, these observations suggest that β_2M may play a role in modulating lymphocyte proliferation, possibly through modification of the CD69 molecule.

Key words: β_2-microglobulin; mononuclear cells; proliferation; CD69.

Introduction

β_2-Microglobulin (β_2M), the light chain of the class I HLA molecule, is a simple polypeptide chain of 99 amino acids. The human gene for it is located in the long arm of chromosome 15[4]. The synthesis of β_2M is regulated at the transcriptional level by interferon. Binding of interferon to its receptors causes the activation of several genes, including those encoding HLA class I and β_2M, resulting in increased synthesis and release of β_2M. The newly synthesised β_2M is complexed with HLA heavy chain in the endoplasmic reticulum[14]. A large excess of free β_2M has been described in the cytoplasm in several cell lines. Lymphoid cells have the greatest density of β_2M on their surface. Activated T cells release more β_2M than activated B cells[9]. After lymphocyte activation, β_2M is released in greater amounts than by resting cells. However, in B cells the rate of synthesis of β_2M is not correlated with immunoglobulin generation[7]. In addition, activation, of polymorphonuclear neutrophils resulted in the release of 2/3 of the cell β_2M content[1]. It has been shown that *in vitro* phytohemagglutinin-stimulated lymphocyte cultures secreted β_2M into the medium[7].

Serum β_2M levels were seen to be elevated in various diseases including lymphoma, myeloma, leukemia,

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chronic inflammation and viral infections[13]. The kidney has been identified as the major site of catabolism of β_2M. In chronic renal failure, β_2M serum levels rise with progressive loss of renal function and peak at about 50 mg/l in the anuric patient[18].

Previously we showed that β_2M had a stimulatory effect on IgM synthesis *in vitro*[12].

The present study addresses the possible influence of native β_2M on T lymphocyte activation *in vitro*. We examined the effect of β_2M on T cell proliferation and expression of the CD69 molecule on lymphocytes. CD69, a type II membrane protein belonging to C-type lectin, is an early activation antigen transiently expressed on T cells through signals via T cell receptor (TCR) cross-linking[16] and involved in TCR/CD3 signaling[15]. It appears that the key event responsible for CD69 induction in lymphocytes is the activation of RAS oncogene[6].

Materials and Methods

A group of 17 healthy volunteers, 7 males and 10 females aged 18–43 years, were examined. Subjects receiving any medication were excluded.

Peripheral venous blood was drawn and anticoagulanted with 20 μ/ml of preservative-free heparin (Sigma). The blood was diluted twice with Hanks' balanced solution and peripheral blood mononuclear cells (PBMCs) were separated on a Histopaque solution gradient (Sigma). The cells were washed 3 times and resuspended in culture medium.

Flow cytometry. Analysis of cell surface marker expression was performed by flow cytometry using a FACSCalibur (Becton Dickinson). The lymphocyte PBMC were specifically analyzed by selective gating based on the parameters of forward and side scatter. The percentage of cells positive for green (FITC) and red (PE) fluorescence was calculated using the Cell Quest program (Becton Dickinson).

Monoclonal antibodies (mAbs) used were: anti-CD69--FITC (Becton Dickinson) and anti-CD3-PE (Becton Dickinson) FITC. PE directly labeled mAbs (IgG1-FITC and IgG1-PE (Becton Dickinson)) were used as negative controls to verify the staining specificity[5].

Proliferation assays. T cells (10^5/well) were cultured in coated microtiter plates. The flat-bottomed microtiter plates (Nunc) were first incubated with mAb OKT3 (Ortho) 1 μg/ml overnight at 4°C in PBS. Unbound antibody was then removed and the ECM proteins (collagen I – COL I, collagen IV – COL IV, fibronectin – FN; Sigma) were incubated in appropriate wells for 3 h at room temperature. Plates were subsequently washed 3 times for 4 days. During the last 16 h of the incubation the cultures were pulsed with 1 μCi of [^{3}H]thymidine. The results are expressed as the arithmetic mean cpm of triplicate cultures[8].

Results

β_2M enhanced CD69 molecule expression on T cells in a dose-dependent manner until a level of 30 mg β_2M/dl was reached. The CD69 expression was enhanced to around 15 times above the control level (1.52 ± 1.03%, 16.78 ± 11.15% and 33.211 ± 20.26% in the control group, 5 mg β_2M/dl group and 30 mg β_2M/dl group, respectively). Further increment of β_2M up to a concentration of 80 g/dl did not enhance the CD69 molecule expression (17.58 ± 10.20%) (Fig. 1).

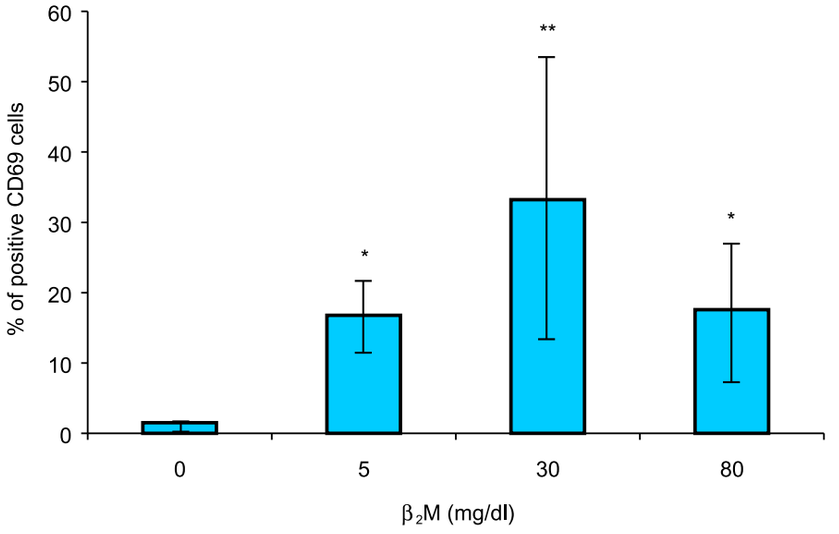
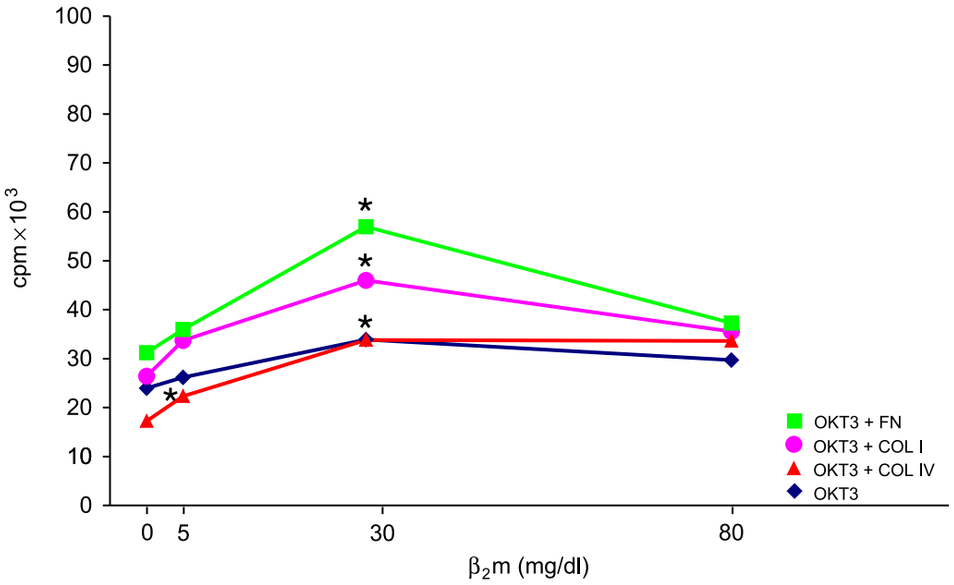


Fig. 1. Concentration-dependent effect of preincubation with β_2M on CD69 expression by PBMC. T cells were incubated (10^5 cells per well) with increasing concentrations of β_2M. After 24 h of stimulation, cells were harvested and assessed for CD69 by flow cytometry. Results are expressed as mean ± SD. One representative experiment out of 9 performed is shown. *$p<0.01$; **$p<0.002$ as compared with β_2M level 0



	β2M (mg/dl)			
	0	5	30	80
OKT3	23 971±16 320	26 157±15 777	33 895±30 042	29 698±13 723
OKT3 + COL I	26 422±21 344	33 726±27 615	46 006±40 481	35 562±30 747
OKT3 +COL IV	17 245± 8 054	22 321±14 105	33 813±28 780	33 622±25 411
OKT3 +FN	31 212±20 290	35 961±24 379	56 967±35 333	37 265±23 683

Fig. 2. The lymphoproliferative responses of T cells isolated from peripheral blood to plate-bound with OKT3 alone (◆) and costimulated with COL IV (▲), with COL I (●), or FN (■) in the presence of increasing concentrations of β_2M. The cells were incubated for 4 days at 37°C. During the last 16 h of the assay the cultures were pulsed with 1 µCi of [^{3}H]thymidine. The results are expressed as mean ± SD. One representative experiment out of 17 performed is shown. *$p<0.05$ as compared with β_2M concentration 0

β_2M and lymphoproliferative responses of T cells stimulated with OKT3 and with ECM proteins has been studied. Stimulation with OKT3 + FN in combination with 30 mg β_2M/dl resulted in a two-fold increase of cell proliferation. When the concentration of β_2M was 80 mg/dl, the effect was no longer observed. A similar effect was observed, when OKT3 and COL I were applied as well as when OKT3 and COL IV were used for the stimulation of T cells. When OKT3 alone was introduced, β_2M revealed no significant effect on T cell proliferation (Fig. 2).

Discussion

We present data that *in vitro* β_2M plays a role in the regulation of the inflammatory process. *In vitro* β_2M at a concentration 30 mg/l showed significant mitogenic activity in lymphoproliferative responses when lymphocytes were stimulated with OKT3 and costimulated with the extracellular proteins collagen or FN.

In accordance with the increased proliferation capability, a significant expression of the CD69 molecule was observed. CD69, together with a subunit of the receptor for interleukin 2 (IL-2) (CD25) and transferin receptor (CD71) are surface molecules called together cell activation markers[2]. CD69 is constitutively expressed on CD3 thymocytes, platelets, Langerhan's cells and bone marrow myeloid precursors. Lymphocytes, natural killer cells, neutrophils and eosinophils express CD69 only when stimulated.

Human T cells as well as B cells can be induced to proliferate by CD69 stimulation in the presence of phorbol myristate acetate (PMA)[19]. CD69 stimulation in the presence of PMA induces transient c-fos gene expression and the formation of specific AP-1 DNA--binding complex in T cells[17]. The induction of AP-1--following CD69 activation with PMA participates in the transcriptional regulation of IL-2 gene expression in T lymphocytes[3]. In T cells, surface CD69 molecules are detectable 2–3 h after TCR/CD3 stimulation and reach a peak level after 18–24 h and decline within the next 2–4 days[16].

Our data indicate that, *in vitro* increasing the level

of β_2M in the presence of co-stimulatory signals coming from the extracellular matrix induces increased lymphocyte proliferation. Increased CD69 marker on lymphocytes is correlated with an accelerated proliferation capability of the cells. It seems that this is an effect of native β_2M. Although it is known that AGEs-β_2M[18] or Des-Lys$^{58}\beta_2$M[11] are highly biologically active, in the performed study native β_2M was applied.

Our data are in accordance with previously demonstrated observation that the supernatants of MLC exposed to β_2M released the increased amount of IL-2 as compared to non-stimulated controls[11].

It is worth to mention, that β_2M serum concentration in kidney insufficiency reaches the level of 50–70 mg/dl, so in the range of concentration β_2M used in the presented study.

Together, these observations suggest, that native β_2M may play a role in modulating lymphocyte proliferation, possibly through modification of CD69 molecule expression.

References

1. Bierrum Ole W., Bierrum Ole J. and Borregaard N. (1987): β_2-Microglobulin in neutrophils: an intragranular protein. J. Immunol., **138**, 3913–3917.
2. Crabtree G. R. (1989): Contingent genetic regulatory events in T lymphocyte activation. Science, **243**, 355–361.
3. D'Ambrosio D., Trotta R. and Vacca A. (1993): Transcriptional regulation of interleukin-2 gene expresion by CD69-generated signals. Eur. J. Immunol., **23**, 2993–2997.
4. Faber H. E., Kucherlapati R. S., Poulik M. D., Ruddle F. H. and Smithies O. (1976): β_2M locus on human chromosome 15. Somat. Cell Genet., **2**, 141–153.
5. Fiszer U., Korczak-Kowalska G., Palasik W., Korlak J., Górski A. and Członkowska A. (1998): Increased expression of adhesion molecule CD18 (LFA-1β) on the leukocytes of peripheral blood in patients with acute ischemic stroke. Acta Neurol. Scand., **97**, 221–224.
6. Górski A., Piekarczyk J., Skurzak H., Nowaczyk M., Dybowska B., Stępień-Sopniewska B., Korczak-Kowalska G., Pazdur J. and Filipowicz-Sosnowska A. (1993): Abnormalities in T cell interactions with the extracellular matrix proteins in a patient with Wegener's granulomatosis. Clin. Immunol. Immunopathol., **69**, 149–154.
7. Hütteroth T. H., Cleve H., Litwin S. D. and Poulik M. D. (1973): The relationship between β_2-microglobulin and immunoglobulin in cultured human lymphoid cell lines. J. Exp. Med., **137**, 838–843.
8. Jerzak M. and Górski A. (1998): T cell adhesion to the extracellular matrix proteins as a determinant of pregnancy success or failure. Immunology, **63**, 135–140.
9. Kin K., Kasahra T., Itoh Y., Sakurabayashi I., Kawai T. and Morita M. (1979): β_2-microglobulin production by highly purified human T and B lymphocytes in cell culture stimulated with various mitogens. Immunology, **36**, 47–54.
10. Miyata T., Lida Y., Ueda Y., Shinzato T., Seo H., Monnier V. M., Maeda K. and Wada Y. (1996): Monocyte/macrophage response to β_2-microglobulin modified with advanced glycation and products. Kidney Int., **49**, 538–550.
11. Nissen M. H. and Claësson M. H. (1987): Proteolytically modified human β_2-microglobulin augments the specific cytotoxic activity in murine mixed lymphocyte culture. J. Immunol., **139**, 1022–1029.
12. Pączek L., Czarkowska B., Schaefer L., Schaefer R. M. and Heidland A. (1992): Effect of β_2-microglobulin on immunoglobulin production. Immunol. Lett., **33**, 87–92.
13. Revillard J. P., Vincent C. and Ramackers J. M. (1989): β_2-Microglobulin: structure, metabolism and alterations in disease. In Gejyo F., Brancaccio D. and Bardin T. (eds.): Dialysis amyloidosis. Wichting Editore, Milano, 7–33.
14. Sege K., Rask L. and Peterson P. A. (1981): Role of β_2M in the intracellular processing of HLA antigens. Biochemistry, **20**, 4523–4530.
15. Swat W., Dessing M., Von Boehmer H. and Kiesielow P. (1993): CD69 expression during selection and maturation of $CD4^+$ $CD8^+$ thymocytes. Eur J. Immunol., **23**, 739–743.
16. Testi R., Philips J. H. and Lanier L. L. (1989): Leu 23 induction as an early marker of functional CD3/T cell antigenic receptor triggering: requirement for receptor cross-linking, prolonged elevation of intracellular (Ca^{2+}) and stimulation of protein kinase. C. J. Immunol., **142**, 1854–1860.
17. Tugores A., Alonso M. A., Sanchez-Madrid F. and De Landazuri M. O. (1992): Human T cell activation through the activation-inducer molecule/CD69 enhanced the activity of transcription factor AP-1. J. Immunol., **148**, 2300–2306.
18. Vincent C., Revillard J. P., Galland R. and Traeger J. (1978): Serum β_2M in hemodialyzed patients. Nephron, **21**, 260–268.
19. Yokoyama W. M., Koning F. and Kehn P. J. (1988): Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. J. Immunol., **141**, 369–376.

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