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ARTICLE

Complement Synthesis Influencing Factors Produced by Acute Myeloid Leukemia Blast Cells

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In a previous study, we found hypercomplementaemia in the sera of acute myeloid leukemia patients. In this study we show that the supernatants of mononuclear cells, derived from peripheral blood taken in the blastic phase, from patients with acute myeloid leukemia (CM-AML) increased the *in vitro* complement protein synthesis of HepG2 hepatocellular carcinoma cells. This effect of CM-AML was mediated by heat labile soluble factors and involved the synthesis of mRNA and protein. Inhibition experiments with anti-cytokine antibodies and immunoaffinity chromatography revealed that this effect of CM-AML is mostly mediated by IL-1 and IL-6. (Pathology Oncology Research Vol 1, No1, 54–59, 1995)

Key words: AML, complement factor B, C1-INH, C3, C4, IL-1b, IL-2, IL-6, TNF

Introduction

Hypercomplementaemia, or the elevation of the levels of over-all haemolytic activity of complement pathways and/or levels of complement components, above the upper limit of normal values, is frequently observed in malignant diseases.[1-6]

In a previous study we have carried out measurements of complement activity in patients with acute myeloid leukemia (AML) and found that both the classical and the alternative pathway showed increased haemolytic activity and the haemolytic C4 titer and the serum level of C3 and factor B components were also increased as compared to those parameters in healthy blood donors .[7] In the sera of acute leukemia patients, taken in the blastic phase, the haemolytic activity of the classical pathway and the level of factor B was significantly elevated compared to those parameters in remission.

As we have shown earlier[8] AML tumor cells produce factors that can increase complement protein synthesis by monocytes and cells of hepatocyte origin *in vitro*. In monocyte cultures the conditioned media of acute myeloid leukemia blast cells (CM-AML) increased C2 and factor B levels and decreased that of C1-esterase inhibitor (C1-INH). This study also shows that CM-AML increased factor B synthesis both at mRNA and protein level in HepG2 hepatocellular carcinoma cells. The complement synthesis enhancing potential of the supernatant of AML blast cells has also been demonstrated on fibroblasts: it substantially increased the factor B synthesis as measured by biosynthetic labelling, it did not affect, however, the production of C1-INH.[9]

The main source of the synthesis of complement proteins in the human body are the hepatocytes of the liver,[10] therefore we chose a well established hepatic cell line, HepG2[11] as a test system to further characterise the factor(s) produced by AML blasts. Here we report, that the factor B production increasing effect of CM-AML involves the synthesis of new mRNA and protein molecules and is mediated mostly by IL-1 and IL-6 released by the mononuclear cells of AML patients.

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Abbreviations: IL-1-: interleukin-1-beta; IL-2: interleukin-2; IL-6: Interleukin-6; TNF: tumor necrosis factor; C2: complement factor 2; C3: complement factor 3; C4: complement factor 4; C1-INH: C1-esterase inhibitor; AML: acute myeloid leukemia; CM-AML: conditioned medium of mononuclear cells derived from the blood of a patient with AML; CM-PBMNC: conditioned medium of peripheral blood mononuclear cells derived from the blood of a healthy volunteer.

Materials and Methods

Reagents

Rabbit anti human C4 IgG was obtained from DAKO (Glostrup, Denmark); goat anti human factor B, C1-INH and C3 IgG and Calibrator 4 from Atlantic Antibodies (Stillwater, MN, USA). Heparin from Richter (Budapest, Hungary). HEPES, Cycloheximide, actinomycin D, amphotericin B and peroxidase labeled streptavidin were purchased from Sigma (St Louis, MO, USA). RPMI 1640 powder, FBS, glutamine and trypsin-EDTA from Flow Laboratories (Irvine, Scotland). Kanamycin from Serva (Heidelberg, Germany). Ficoll and CNBr activated Sepharose 4B from Pharmacia (Uppsala, Sweden). Uromiro 75% from Bracco (Milano, Italy).

Preparation of conditioned media

Heparinized (5 U/ml) peripheral blood was taken with informed consent from three freshly diagnosed patients with acute myeloid leukemia (AML) in the Haematology Department of our Institute during the overt leukemic phase, when more than 90% of the cells in the periphery were tumor cells. According to FAB classification, patients K, F, and P belonged to class M2, M4, and M5, respectively. Diagnosis was established on the basis of morphology, cytochemistry and surface markers. Mononuclear cells were separated by the standard Ficoll-Uromiro method (r=1.077 g/cm^3), washed three times with RPMI 1640, and then after adjusting the cell count to 10^6/ml they were cultured in RPMI 1640 containing 24 mM $NaHCO_3$ and 20 mM HEPES, supplemented with 10% heat inactivated fetal bovine serum, glutamine, 20 µg/ml kanamycin and 2.5 µg/ml amphotericin B (referred below as culture medium) for three days at 37°C. Mononuclear cells from patient K were cultured in RPMI-1640, glutamine, antibiotics. The lack of serum proteins apparently did not influence the viability of cells. Then supernatants were harvested on the third day, centrifuged, sterile filtered (Minisart 0.22 m, Sartorius, Goettingen, Germany) and stored in aliquots at -20°C until used.

Conditioned media of the peripheral blood mononuclear cells of healthy adults (CM-PBMNC) were obtained and processed the same manner as CM-AML.

Cell cultures used for measurement of complement production

The human hepatoblastoma cell line, HepG2,[11] was maintained in RPMI 1640 containing 24 mM $NaHCO_3$ and 20 mM HEPES, supplemented with 10% heat inactivated FBS, 2 mM Glutamine, 20 µg/ml kanamycin and 2.5 µg/ml amphotericin B and kept at 37°C in 5% CO_2, in 250 cm^2 culture flasks (Greiner, Frickenhausen, Germany).

For the experiments, cells were trypsinized and 200 µl cell suspension was seeded into the wells of 96-well tissue culture plate (Becton Dickinson, Lincoln Park, NJ, USA) at a density of 2-3 x 10^5 cells per ml. Cultures were treated on the next day with CM-AMLs (1:2 dilution with raw culture medium) for 24 hours then supernatants harvested and stored at -20°C until tested for the level of complement proteins.

In the case of long term experiments, cells were washed twice with culture medium at the end of the first 24 hrs culture period and then cultured for further 24 hrs and supernatants were then harvested.

Cultures were inspected before and at the termination of experiments through an inverted light microscope.

Assay of complement proteins

Factor B, C1-INH, C3 and C4 concentrations in the supernatants of HepG2 cultures were measured by double polyclonal sandwich ELISA. Briefly, flat bottomed 96 well microtiter plates (Greiner) were coated by the respective antibody preparation (IgG fraction), blocked then standard (serial dilutions of Calibrator 4) and the samples were applied on the plate. The captured antigen was detected by the same antibody labeled with horse radish peroxidase. In the case of C1-INH biotinylated antibody and streptavidin-peroxidase conjugate was used. The colorigenic reaction was performed with TMB/ureaperoxide or OPD/ureaperoxide substrate (measured at 450 nm/620 nm or 492 nm/620 nm, respectively, on an Anthos II ELISA plate reader). The detection limit of the tests were 2 ng/ml for factor B, 100 pg/ml for C1-INH, 10 ng/ml for C3 and 2 ng/ml for C4.

Heat inactivation of CM-AMLs

Aliquots of CM-AMLs were submerged in boiling water for 10 minutes, the spun down, sterile filtered and diluted to 1:2 with culture medium and added to HepG2 cultures with suitable controls.

Cytokine inhibition experiments

Anti-cytokine antisera or purified IgG antibodies were added to CM-AMLs, incubated for 30 minutes at 37°C then brought to HepG2 cell cultures. Supernatants were harvested next day and factor B levels determined.

Anti-cytokine antisera or antibodies were kindly provided or purchased as follows: rabbit anti-human interleukin-2 IgG (<1µg/ml neutralises 1 U/ml IL-2 in biological assay, 1 mg/ml, used in 1:100) (Boehringer Mannheim); mouse monoclonal anti-human interleukin-6 IgG (20 pg neutralises 1 U IL-6 in biological assay, 1 mg/ml, used in 1:10,000) (Biosource International); goat anti-human interleukin-1-beta IgG (10 µg/ml neutralises 50 pg/ml IL-1~ in biological assay, 1 mg/ml, used in 1:1000) (British Biotechnology); rabbit anti-human tumor necrosis factor antiserum (10,000 neutralising U/ml, used in 1:1000), Immunogenetics; anti-TNF rabbit antiserum, (Dr Michael Kirschfink, Johannes Gutenberg Universität, Heidelberg, Germany;) rabbit anti-human

TNF IgG (70.000 neutralising U/ml, used 1:100), (Dr Ernő Duda, Biological Research Center, Szeged, Hungary;) rabbit anti-IL-6 antiserum, (Dr András Falus, Semmelweis Medical University, Budapest, Hungary.)

Affinity chromatography of CM-AMLs

Anti-cytokine antibodies were coupled to CNBr activated Sepharose 4B beads using the standard method recommended by the manufacturer. Gels were transferred into Biospin chromatography columns (BioRad) and extensively washed with 3.5 M $MgCl_2$, 100 mM phosphate buffered saline (pH 7.4) and culture medium, then CM-AML was applied. Columns were centrifuged with approximately 100 g for 10 seconds following each step. After the adsorption step CM-AMLs were sterile filtered and diluted to 1:2 with culture medium.

Statistical analysis of data was performed with Student's t test using a commercial statistical software (STATGRAFICS, STSC, Inc., Rockville, MD, USA).

Results

The effect of CM-AMLs on complement component production by HepG2 cells

Incubation of HepG2 cells with CM-AMLs (diluted 1:2) resulted in marked elevation in factor B and C1-INH concentrations in the culture supernatants *(Table 1)*. The increased levels of factor B and C1-INH persisted even after the removal of CM-AMLs: the effect of CM-AML on C1-INH level in the second 24 hrs seemed to be more pronounced than the effect exerted on factor B level. Changes in the amounts of C4 released were not consequent for all CM-AMLs in the first 24 hrs and no effect was found after the removal of CM-AMLs. Interestingly, we found a significant increase as compared to the medium control in the level of C3 after the removal of CM-AMLs, while no significant effect was detected in the first 24 hrs.

Table 1. The effect of CM-AMLs on complement component production by HepG2 cells

1st 24hrs		*CM-AML K*	*CM-AML F*	*CM-AML P*
Factor B	–	72.8 ± 9.1	57.9 ± 17.0	44.3 ± 3.3
	+	126.6 ± 30.9*	150.3 ± 58.8*	155.5 ± 21.8*
C3	–	594.6 ± 98.1	576.2 ± 59.0	470.6 ± 119.9
	+	517.1 ± 49.5 NS	611.8 ± 93.7 NS	607.7 ± 169.9 NS
C4	–	114.6 ± 6.2	113.8 ± 9.3	96.4 ± 17.8
	+	101.7 ± 13.1*	119.4 ± 12.6 NS	133.8 ± 33.7*
C1-INH	–	426.4 ± 31.8	302.0 ± 101.4	ND
	+	557.0 ± 64.3*	542.9 ± 184.3*	ND
2nd 24 hrs				
Factor B	–	52.8 ± 5.6	33.8 ± 14.1	41.9 ± 19.5
	+	85.5 ± 13.4*	51.3 ± 11.8*	70.0 ± 15.3*
C3	–	334.0 ± 75.2	321.6 ± 37.2	481.0 ± 93.0
	+	411.7 ± 37.7*	414.3 ± 20.4*	633.8 ± 96.5*
C4	–	89.0 ± 11.6	89.8 ± 12.8	126.7 ± 36.0
	+	85.7 ± 9.4 NS	84.9 ± 14.6 NS	131.3 ± 41.3 NS
C1-INH	–	435.8 ± 44.8	292.7 ± 152.8	ND
	+	815.0 ± 245.0*	669.1 ± 396.5*	ND

HepG2 cell cultures (4-6 x 10^4 cells in 200 µl culture medium) established on the previous day were treated overnight with CM-AMLs (diluted 1:2 with culture medium and sterile filtered). Next day supernatants were harvested and cells washed twice with culture medium then fresh medium was added to each well and cells were cultured for an additional 24 hours (complement protein levels are presented, mean ± SD, data of two or three experiments, done in duplicates or triplicates).

Doses: ng/ml for Factor B, C3 and C4; pg/ml for C1-INH

– : medium control; + : cultures treated with CM-AML, respective

* $p < 0.05$; NS: non significant; ND: not determined

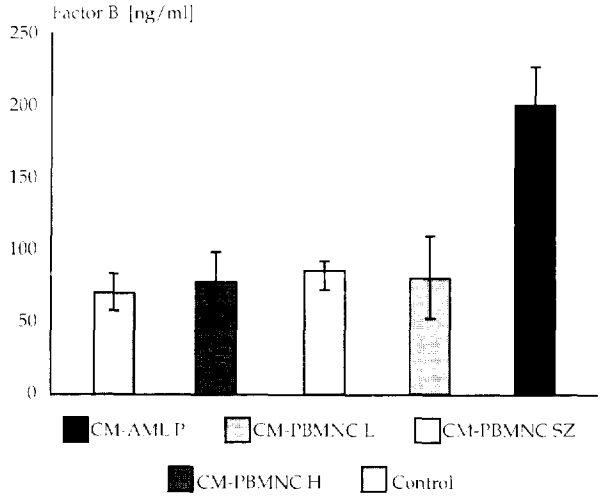


Figure 1. HepG2 cell cultures established on the previous day were treated with the supernatant of peripheral blood mononuclear cells of healthy volunteers (CM-PBMNCs) and that of an acute myeloid leukemia patient (CM-AML P) for 24 hrs. Supernatants were then harvested and factor B levels determined. Mean data of three independent experiments, each done in duplicates.

CM-AMLs themselves contained low levels of all complement proteins tested (e.g. for factor B: CM-AML K and F was below the detection limit, CM-AML P 7.8 ng/ml).

Effect of control conditioned media

Culture supernatants of peripheral blood mononuclear cells from healthy volunteers (CM-PBMNC) were obtained exactly the same manner as the CM-AMLs and tested for their ability of stimulating factor B release by HepG2 cells. No effect of CM-PBMNCs on the factor B production by HepG2 was observed *(Fig.1)*. CM PBMNC L, SZ and H contained 8.6, 6.0 and 5.6 ng/ml factor B, respectively.

Heat inactivation of CM-AMLs

CM-AMLs were exposed to 100°C for ten minutes, then sterile filtered, diluted to 1:2 and added to HepG2 cultures. Heat treatment of CM-AMLs resulted in the complete loss of activity as assessed by the factor B production of HepG2 cells *(Fig.2)*.

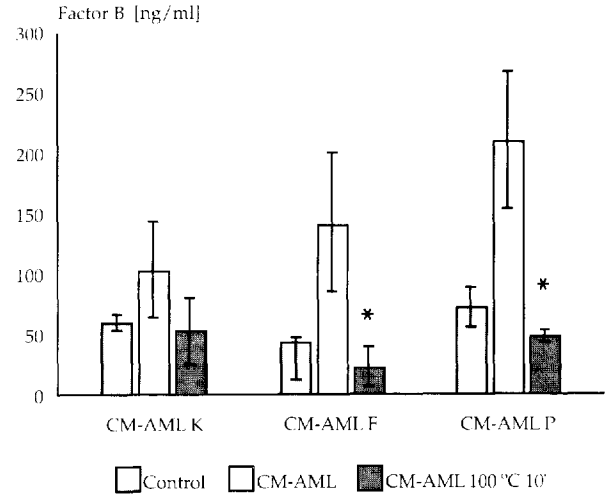


*Figure 2. HepG2 cell cultures established on the previous day were treated overnight with CM-AMLs and the same CM-AMLs subjected to heat treatment. Supernatants were collected next day and factor B levels determined. Mean of three experiments. * $p < 0.01$ heat treated vs not treated*

Inhibition of the effect of CM-AMLs with mRNA and protein synthesis inhibitors

HepG2 cultures were treated with CM-AMLs containing mRNA synthesis inhibitor actinomycin D (0.01-0.05 mg/ml, 7.9 nM-0.4 mM) or protein synthesis inhibitor cycloheximide (0.1-0.5 mg/ml, 0.4-1.8 mM) and suitable controls for 24 hours. The addition of these inhibitors to CM-AMLs markedly reduced the factor B production stimulating effect of all conditioned media *(Fig.3.)*.

The effect of the addition of anti-cytokine antibodies on the factor B production stimulating activity of CM-AMLs

HepG2 cultures were treated for 24 hrs with CM-AMLs containing anti-cytokine antisera or antibodies in dilutions indicated in Materials and Methods and factor B levels in the culture supernatants were determined *(Table 2)*. The addition of anti-IL-1 or anti-IL-6 antibodies markedly reduced the activity of CM-AMLs. The concomitant addition of both antibodies resulted in further inhibition in the case of CM-AML F. The addition of anti-IL-2 did not significantly influence the effect of CM-AMLs on factor B synthesis by HepG2 cells. The experiments with anti-TNF antibodies and antisera did not give clear-cut results. In part of the experiments the addition of anti-TNF antibodies/antiserum resulted in marked inhibition of factor B stimulation, but this effect could not be consistently demonstrated. None of the anti-cytokine antisera or antibodies alone (diluted to the same concentration in culture medium) had remarkable effects on factor B levels in HepG2 cultures and they did not interfere with the factor B assay.

***Table 2.* Modulation of the effect of CM-AMLs with the addition of anti-cytokine antibodies or antiserum**

	CM-AML K	*CM-AML F*	*CM-AML P*
none	55.5 ± 23.4	120.1 ± 87.8	146.5 ± 8.3
+ αIL-1-β	10.6 ± 9.7*	50.7 ± 27.3	74.1 ± 15.9*
none	55.5 ± 23.4	120.0 ± 87.5	140.4 ± 20.9
+ αIL-2	45.8 ± 28.2	121.6 ± 86.6	100.7 ± 31.8
none	51.0 ± 18.5	109.3 ± 69.0	146.5 ± 8.3
+ αIL-6	4.6 ± 9.7*	37.8 ± 24.4	112.9 ± 23.3*
none	66.1 ± 7.6	161.8 ± 76.3	140.3 ± 20.7
+ αTNF	56.7 ± 27.0	99.2 ± 65.8	130.1 ± 22.7
none	51.0 ± 18.5	109.3 ± 69.0	152.5 ± 10.9
+ αIL-1-β & αIL-6	5.7 ± 15.0*	21.9 ± 15.6*	85.0 ± 42.8*

HepG2 cells were incubated with CM-AML containing anti-cytokine antibodies or antiserum and suitable controls for 24 hours, then supernatants were harvested and factor B level determined. Data are presented as elevation in factor B level corrected with medium control, [ng/ml], mean of three experiments ± SD.
* $p < 0.05$

Removal of cytokines from CM-AMLs by affinity chromatography

CM-AMLs were passed through Sepharose 4B columns to which anti-cytokine antibodies were coupled, then transferred to HepG2 cultures. The removal of IL-1 and that of IL-6 resulted in well-measurable inhibition of the activity of CM-AMLs. The removal of IL-2 did not influence the effect of CM-AMLs, whereas equivocal results were obtained in the case of TNF *(Table 3)*.

Discussion

We have shown in a cell line of hepatocellular origin that the conditioned media of acute myeloid leukemia blast cells markedly stimulated the production of factor B

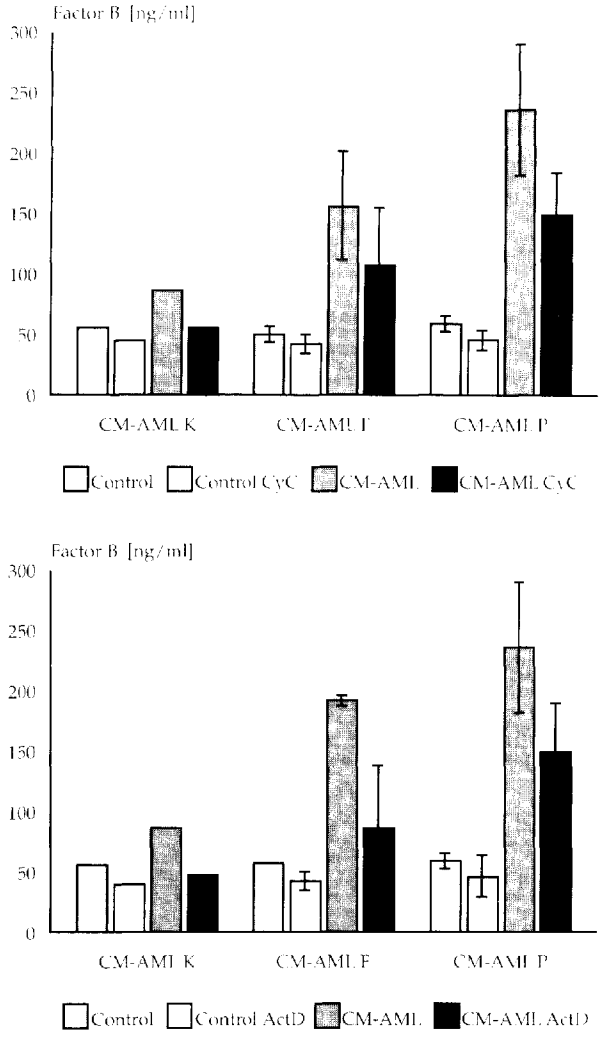
***Table 3.* Modulation of the effect of CM-AMLs by immunoaffinity chromatography**

	CM-AML F	*CM-AML P*
non treated	114.4 ± 38.6	149.1 ± 58.8
+ IL-1-β	73.7 ± 22.9	108.5 ± 63.6
non treated	114.4 ± 38.6	130.6 ± 65.8
+ IL-2	109.7 ± 55.2	115.6 ± 65.0
non treated	126.8 ± 35.7	155.4 ± 48.5
+ IL-6	49.9 ± 19.0*	101.6 ± 38.8
non treated	114.4 ± 38.6	155.4 ± 48.5
+ TNF	99.0 ± 39.6	127.2 ± 51.7

Aliquots of CM-AMLs F and P were passed through Sepharose 4B columns with the appropriate anti-cytokine antibodies bound to the matrix. CM-AMLs were then sterile filtered, diluted to 1:2 with culture medium and transferred to HepG2 cultures with suitable controls. Supernatants were harvested next day and factor B level determined. Data are presented as elevation in factor B level corrected with medium control [ng/ml], mean SD. Data of two or three experiments, done in duplicates or triplicates.
* $p < 0.05$

and caused only minor changes in that of C3 and C4. This is in accordance with our previous *in vivo* results, that serum factor B levels in patients with acute leukemia showed a most significant increase as compared to that of controls, while levels in remission were not different from control values. Factor B levels showed connection with blast cell number in the periphery in as much as they returned to normal after successful chemotherapy. CM-AMLs also markedly stimulated the production of C1-INH by HepG2 cells.

The release of these factors by mononuclear cells of AML patients is most probably not due to bacterial endotoxin (LPS) contamination, since the conditioned media of peripheral blood mononuclear cells, obtained by the same procedure as CM-AML, had no effect on the level of factor B in HepG2 cultures.



Figures 3 a, b. *Modulation of the effect of CM-AMLs by translation and transcription inhibitors. HepG2 cultures treated overnight with CM-AMLs containing cycloheximide (above) or actinomycin D (below). Supernatants were then harvested and factor B levels determined. Mean data of three independent experiments, each done in duplicates. In case of CM-AML K mean of two experiments is presented*

The effect of CM-AMLs on the factor B production by HepG2 cells were due to heat labile factors and involved the synthesis of new mRNA and protein molecules since it could be inhibited by transcription and translation inhibitors.

Gel filtration experiments indicated that the molecular masses of the complement production influencing factors in CM-AML K are between 10 and 50 kD. The same experiments revealed a factor B synthesis increasing component and a decreasing one (data not shown).

AML tumor cells have been shown to synthesise a number of cytokines by several groups[12–14] and the high level of some cytokines in the sera of AML patients has also been documented.[15] Amongst the most readily detected cytokines released by AML blast cells are IL-1, IL-2, IL-6 and TNF.

IL-1 and IL-6 have been shown to have synergistic stimulating effect on factor B production in HepG2 cells both at the mRNA and the protein level.[16] TNF is also capable of influencing the production of factor B and C3 by HepG2 cells.[17]

Using anti-cytokine antibodies or antisera, raised against these cytokines, we have shown in the present study that IL-1 and IL-6 play substantial roles in the *in vitro* factor B production stimulating effect of the conditioned media of acute myeloid leukemic blast cells. The removal of IL-1 or IL-6 from CM-AMLs by immunoaffinity chromatography also confirmed that these factors are present in these myeloid blast cell supernatants and they are responsible for most of the factor B synthesis simulating effect in HepG2 cell cultures.

Same kind of experiments revealed that IL-2 is not involved in the effect of CM-AMLs. The participation of TNF in the factor B synthesis enhancing effect of the CM-AMLs can not be excluded by our results, but most probably its role is secondary to IL-1 or IL-6.

The involvement of IL-1 in the proliferation of AML blast cells have been confirmed in several reports, the addition of IL-1 stimulates the proliferation of AML blasts *in vitro*[18] and the blocking of IL-1 by antibodies or its natural antagonist has an inhibitory role on AML blast proliferation.[19,20] IL-6 has been shown to greatly enhance the GM-CSF-dependent proliferation of the clonogenic cells from several different patients.[21] Because of the above mentioned synergistic effect of IL-1 and IL-6 on the factor B synthesis by HepG2 cells, a well established model system of human hepatocytes, the serum factor B level may well reflect the progression of leukemic blast proliferation in AML patients. High level of factor B and other complement proteins in turn may contribute to the progression of leukemia since hypercomplementaemia was found to be associated with poor prognosis of certain malignant diseases.[22]

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References

1. *Lichtenfeld JL, Wernik PH, Mardiney MR and Zarco RM:* Abnormalities of complement and its components in patients with acute leukemia, Hodgkin's disease and sarcoma. Cancer Res 36:3678-3685, 1976.
2. *Nishioka K, Kawamura K, Hirayama T, Kawashima T, Shimada K and Kogure M:* The complement system in tumor immunity: significance of elevated levels of complement in tumor bearing hosts. Ann NY Acad Sci 276:303-315, 1976.
3. *Pulay ÁT, Füst G and Csömör A:* Serum complement levels in patients with cancer of the uterine cervix before and after radiation therapy. Neoplasma 27:211-216, 1980.
4. *Bach-Mortensen N, Osther K and Stroyer I:* C1-esterase inactivators and C4 in malignant diseases. Lancet 2:499-500, 1975.
5. *Verhaegen H, De Cock W, De Cree J MD and Verbruggen F:* Increase of serum complement levels in cancer patients with progressing tumors. Cancer 38:1608-1613, 1976.
6. *Astrup J, Colstrup H and Frandsen B:* Complement C1-inactivator in the serum of patients with malignant disease. Acta Radiol Ther Phys Biol, 16, Fasc 5:394-400, 1977.
7. *Do-Quang Minh, Czink E, Mód A, Füst G and Hollán SR:* Serial complement measurements in patients with leukemia. Clin Lab Hematol 5:23-24, 1983.
8. *Gyapay G, Schmidt B, Vályi M, Falus A, Anh-Tuan N, Pányi A, Kókai M, Ónody K, Mód A and Füst G:* Effect of Conditioned Media of Acute Myeloid Blast Cells on Complement Synthesis by Cultured Human Cells of Monocyte and Hepatocyte Origin. Complement and Inflammation 8:370-377, 1991.
9. *Schmidt B, Gyapay G, Kramer J and Füst G:* Complement Synthesis Regulating factors in the Conditioned Medium of Acute Myeloid Leukemia (AML) Blast Cells. Mol Immunol 30: (Suppl 1): 50, 1993.
10. *Alper CA, Johnson AM, Birtch AG and Moore FD:* Human C3: evidence for the liver as the primary site of synthesis. Science 163:286, 1969.
11. *Knowles BB, Howe CC and Aden DP:* Human hepatocellular carcinoma cell lines secrete the major plasma proteins and Hepatitis B surface antigen. Science 209:497, 1983.
12. *Kurzrock R, Kantarjian H, Wetzler M, Estrov Z, Estey E, Troutman Worden K, Gutterman JU and Talpaz M:* Ubiquitous expression of cytokines in diverse leukemias of lymphoid and myeloid lineage. Exp Hematol 21:80-85, 1993.
13. *Griffin JD, Rambaldi A, Vellenga E, Young DC, Ostapovicz D and Cannistra SA:* Secretion of interleukin-1 by acute myeloid leukemia cells in vitro induces endothelial cells to secrete colony stimulating factors. Blood 70:218-21, 1987.
14. *Lopez M, Maroc N, Kerangueven E, Bardin F, Courcoul M, Lavezzi C, Birg F and Mannoni P:* Coexpression of the genes for interleukin 6 and its receptor without apparent involvement in the proliferation of acute myeloid leukemia cells. Exp Hematol 19:797-803, 1991.
15. *Cimino G, Amadori S, Cava MC, De Sanctis V, Petti MC, Di Gregorio AO, Sgadari C, Vegna L, Cimino G and Mandelli F:* Serum Interleukin-2 (IL-2), soluble IL-2 receptors and tumor necrosis factor-alpha levels are significantly increased in acute myeloid leukemia patients. Leukemia 5:32-35, 1991.
16. *Falus A, Rokita H, Walcz E, Brozik M, Hidvégi T and Merétey K:* Hormonal regulation of complement biosynthesis in human cell lines – II. Upregulation of the biosynthesis of complement components C3, factor B, and C1 inhibitor by interleukin-6 and interleukin-1 in human hepatoma cell line. Mol Immunol 27:197-201, 1990.
17. *Perlmutter DH, Dinarello CA, Punsal PI and Colten HR:* Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. J Clin Invest 78:1349-1354, 1986.
18. *Wagteveld AJ, Esselink MT, Limburg P, Halie MR and Vellenga E:* The effects of IL-1- and IL-4 on the proliferation and secretion of growth factors by acute myeloblastic leukemic cells. Leukemia 6:1020-1024, 1992.
19. *Estrov Z, Kurzrock R, Estey E, Wetzler M, Ferrajoli A, Harris D, Blake M, Gutterman JU and Talpaz M:* Inhibition of acute myelogenous leukemia blast proliferation by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors. Blood 79:1938-1945, 1992.
20. *Rambaldi A, Torcia M, Dinarello CA, Barbui T and Cozzoloni F:* Modulation of cell proliferation and cytokine production in AML by recombinant interleukin-1 receptor antagonist. Leukemia (Suppl 2): S10-12, 1993.
21. *Hoang T, Haman A, Goncalves O, Wong GG and Clark SC:* Interleukin-6 enhances growth factor-dependent proliferation of the blast cells of acute myeloblastic leukemia. Blood 72:823-26, 1988.
22. *Füst G, Schmidt B and Varga L:* Clinical significance of hypercomplementemia. In: New aspects of complement structure and function. (Erdei A, Ed.), RG. Landes Company, Austin 1994.