Brief Definitive Reports

THE RELATIONSHIP BETWEEN β_2-MICROGLOBULIN AND IMMUNOGLOBULIN IN CULTURED HUMAN LYMPHOID CELL LINES*

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The human protein β_2-microglobulin is present in serum, cerebrospinal fluid, and urine of normal persons and found in increased amounts in the urine of patients with renal tubular disorders (1). Recent studies on the primary structure of β_2-microglobulin have shown extensive homologies with the constant portions of immunoglobulin light and IgG heavy chains, in particular, to the C_H3 domain (2, 3). β_2-microglobulin has been demonstrated on the cell membrane of peripheral blood lymphocytes,[1] and it has been shown that phytohemagglutinin-stimulated lymphocytes in short-term culture secrete β_2-microglobulin into the medium (4).

In the present study, the secretion and membrane expression of β_2-microglobulin and immunoglobulin were analyzed in established human lymphoid cell lines. The results indicate that the cellular expression of these two proteins is independent.

Materials and Methods

Lymphoid cell lines were established and maintained as previously described (5). The lines were kept in exponential growth phase by daily addition of fresh culture medium. Viability, as determined by exclusion of trypan blue, exceeded 95%. Lines PG-LC and SWB-5A were kindly provided by Doctors K. Hirschhorn and P. Glade, Mount Sinai School of Medicine, New York; the others were established in our laboratory.

β_2-microglobulin was purified from the urine of patients with renal tubular dysfunction (6). Antisera to β_2-microglobulin were raised in rabbits and rendered monospecific by absorption with lyophilized human serum, fetal calf serum, lyophilized urinary proteins, and urinary red cell membrane proteins. Anti-immunoglobulin antisera with specificity for κ- and λ-light chains and the Ig classes were prepared in rabbits and appropriately absorbed (5, 7).

β_2-microglobulin was measured in the medium of lymphoid cell lines as follows: 2×10^7 cells from exponentially growing cultures were washed and resuspended in 20 ml of fresh

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[1] Poulik, M. D. 1973. Presence of β_2-microglobulin on B- and T-cells and lymphocytotoxicity of anti-β_2-microglobulin sera. Manuscript submitted for publication.

culture medium and incubated at 37°C for 24 and 48 h, respectively. The cells were separated by centrifugation and the supernatant medium was dialyzed against distilled water, lyophilized, and reconstituted in a volume of 0.3 ml. The concentration of β_2-microglobulin present in these samples was assayed by radial immunodiffusion with purified β_2-microglobulin as standard.

The relationship between β_2-microglobulin and the IgG secreted into the medium was studied by using ^{14}C-radiolabeled L-leucine: 2×10^7 cells were incubated for 24 h in 2 ml of minimal essential medium without L-leucine containing 5% fetal calf serum, 5 μCi L-[^{14}C]-leucine (sp act 45 mCi/mM; New England Nuclear, Boston, Mass.), and antibiotics. The cells were separated from the medium by centrifugation for 2 h at 12,000 rpm at 4°C. The labeled β_2-microglobulin and IgG secreted into the culture fluid were determined by specific immunological precipitation (8). 10 μl of anti-β_2-microglobulin antiserum, 10 μl of anti-IgG, or 10 μl of normal rabbit serum in control samples were added to 100-μl aliquots of culture medium and incubated for 30 min at 37°C. The rabbit Ig was precipitated by incubation with 200 μl of goat anti-rabbit Ig antiserum for 2 h at 37°C. The precipitates were washed four times with cold 0.02 M phosphate-buffered saline, pH 7.2 (PBS), containing 0.05 M L-leucine, subsequently dissolved in 0.6 ml of 0.25 N acetic acid and mixed with 2.4 ml of scintillation fluid (Aquasol; New England Nuclear). Total secreted protein was measured as the radioactivity precipitated by 10% trichloroacetic acid. Radioactivity was measured as counts per minute in a Packard liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

For detection of membrane-associated β_2-microglobulin and Ig, the mixed antiglobulin reaction was employed (5, 9).

Antigenic modulation of cell surface antigens by specific antibody was performed as follows: antisera employed were heat inactivated at 56°C for 30 min, dialyzed against 0.15 M NaCl, and sterilized. Cells were incubated with modulating antisera at 37°C for time periods varying from 15 min to 12 h, and then washed twice with cold PBS, followed by incubation with the test antiserum at 0°C for 1 h (7).

RESULTS

Secretion of β_2-Microglobulin in Established Lymphoid Cell Lines.—β_2-microglobulin was demonstrated in concentrated culture medium from 12 established cell lines by double immunodiffusion. All media gave a single precipitin band, which showed a reaction of identity with purified urinary β_2-microglobulin (Fig. 1 *A*). Concentrated control medium did not react with the antiserum; unconcentrated culture medium produced a slight deflection of the precipitin line. The antiserum did not react with purified monoclonal proteins of IgG, IgA, IgM, and IgD classes (Fig. 1 *B*).

Quantitative data for secretion of β_2-microglobulin were obtained by culturing nine cell lines under the conditions described. The amount of β_2-microglobulin ranged from 0.22 to 0.70 (mean 0.43 $\pm$ 0.16 SD) μg/ml of culture medium per 10^6 cells for 24 h and from 0.42 to 1.21 (mean 0.78 $\pm$ 0.28 SD) μg/ml per 10^6 cells for 48 h. Based on a molecular weight for β_2-microglobulin of 11,600 daltons, the number of molecules secreted by the most active cell line can be calculated to be 21 molecules per cell per second.

Relationship between β_2-Microglobulin and IgG Secretion.—β_2-microglobulin and IgG secretion was studied by specific immunological precipitation of L-[^{14}C]leucine-labeled proteins (Table I). β_2-microglobulin was found in all

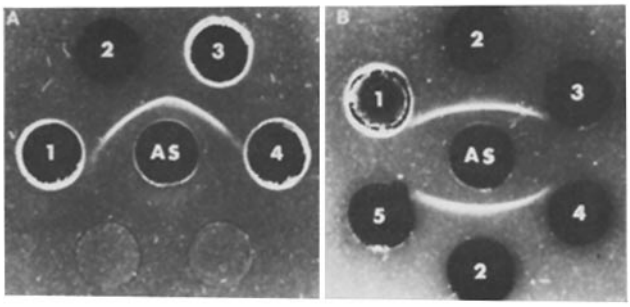

FIG. 1. (*A*) Double immunodiffusion analysis of β_2-microglobulin in culture medium. (*1*) Unconcentrated medium PG-LC; (*2*) β_2-microglobulin, 0.156 mg/ml; (*3*) 40-fold concentrated medium PG-LC; (*4*) 40-fold concentrated control medium; (*AS*) anti-β_2-microglobulin antiserum. (*B*) Double immunodiffusion analysis of anti-β_2-microglobulin antiserum (*AS*). (*1*) IgD; (*2*) β_2-microglobulin, 0.156 mg/ml; (*3*) IgG1; (*4*) IgM; (*5*) IgA. Concentration of monoclonal proteins 2 mg/ml.

TABLE I

Relationship between β_2-Microglobulin and IgG Secretion as Measured by Specific Immunological Precipitation in [C^{14}]Leucine-Labeled Cultures

Cell line	Specific immunological precipitation*				Nonspecific precipitation Normal rabbit serum	TCA precipitation
	β_2-microglobulin		IgG			
	cpm	Total protein	cpm	Total protein		
		%		%	*cpm*	*cpm*
WO	335	3.0	5,774	52	136	10,985
CL	394	0.89	4,035	9.2	423	43,985
CS	617	2.4	9,432	37	424	25,551
MW	456	3.1	5,327	37	242	14,453
PG-LC	373	2.3	651	4.0	300	16,023
HH	527	2.4	679	3.2	224	21,306

* Counts per minute in specific precipitates; nonspecific precipitation by normal rabbit serum has been subtracted.

cell lines; the amounts produced were relatively uniform and represented 0.89–3.1% of total secreted proteins. In contrast, IgG secretion varied widely and ranged from 3.2 to 52% of trichloroacetic acid-precipitable counts. Control experiments confirmed the specificity of the assay systems: precipitation of radioactive material by anti-β_2-microglobulin antiserum was blocked by 100 μg of β_2-microglobulin but not by 100 μg of IgG. Precipitation by anti-IgG antiserum was blocked by 100 μg of IgG but not by 100 μg of β_2-microglobulin.

Membrane Expression of β_2-Microglobulin and Immunoglobulin.—The cell lines studied were previously characterized for the membrane-associated Ig (MAIg). κ and μ were the only Ig antigens detected by the mixed anti-

globulin reaction (5). Using a modification of this technique β_2-microglobulin could be detected on all nine cell lines examined; 13–51% of cells formed rosettes (Table II). Control experiments established specificity of the detection system: 5 μg of β_2-microglobulin inhibited rosette formation, whereas 100 μg of monoclonal proteins of IgG subclasses 1–4, IgA, IgM, IgD, and isolated κ- and λ-light chains failed to inhibit. No correlation was apparent between membrane expression of Ig antigens and β_2-microglobulin (Table II). Further, no correlation was observed between the percentage of cells carrying β_2-microglobulin on their surface and the amounts detected in the culture medium.

Previous studies had demonstrated that MAIg were lost from the cell surface after incubation with specific antiserum (7). Modulation experiments were designed to examine the membrane relationship between MAIg and

TABLE II

Membrane Expression of κ-, μ-, and β_2-Microglobulin Antigens

Cell line	Rosettes		
	κ	μ	β_2-microglobulin
		%	
HG	42	35	13
TM	37	15	22
MW	46	35	19
JK	48	41	30
HH	24	0	51
CL	13	14	41
WO	13	60	49
PG-LC	54	44	48
SWB-5A	25	31	37

β_2-microglobulin (Table III). Anti-κ antiserum led to disappearance of κ-antigen from the cell surface, anti-μ antiserum to disappearance of μ-antigen. In contrast, exposure of cells to anti-β_2-microglobulin antiserum under these conditions had no effect on the percentage of cells bearing β_2-microglobulin. Anti-κ and anti-μ antisera had no influence on the expression of membrane β_2-microglobulin, nor had antiserum to β_2-microglobulin any effect on the expression of κ- or μ-membrane antigens. The experiments also included very brief exposure times to anti-β_2-microglobulin antiserum from 15 to 30 min, long exposure times from 3 to 12 h, and exposure to various antiserum concentrations. All these controls failed to demonstrate modulation of membrane β_2-microglobulin. In other experiments cross-linking was enhanced by using a second layer of goat anti-rabbit Ig antiserum; this treatment also failed to produce modulation of β_2-microglobulin.

TABLE III

*Effect of Anti-κ, Anti-μ, and Anti-β_2-Microglobulin Antiserum on the Expression of κ-, μ-, and β_2-Microglobulin Antigen**

Incubation with specific antiserum for modulation	Test system for membrane antigens	Rosettes
3 h, 37°C	*1 h, 0°C*	%
–	κ	67
–	μ	45
–	β_2	62
Anti-κ	κ	7
"	β_2	54
Anti-μ	μ	3
"	β_2	54
Anti-β_2	β_2	66
"	κ	45
"	μ	51

* Cell line PG-LC; antisera dilutions: anti-κ/20, anti-μ/5, anti-β_2/10. Cells were washed twice between first and second incubation.

DISCUSSION

The present study demonstrates that cultured human lymphoid cells have β_2-microglobulin on their surface and actively secrete this protein into the culture medium. These results extend the recent observation that β_2-microglobulin is a surface marker of both B and T lymphocytes.[1]

The findings permitted comparison of the membrane expression of β_2-microglobulin and Ig. Whereas Ig surface receptors are readily modulated after exposure to specific antisera, no modulation of β_2-microglobulin was observed. Antigenic modulation has been used previously to demonstrate the relationship between cell surface antigens (7, 10). The results outlined in Table III fail to support a topographical relationship between surface β_2-microglobulin and Ig.

The secretion of the two proteins into the medium did not parallel each other. This finding while valid for the cultured lymphoblastoid cell does not exclude the possibility that cells in other phases of the immune response may have synthetic activities correlated to β_2-microglobulin secretion.

SUMMARY

β_2-microglobulin was detected on the cell surface and in the medium of human lymphoid cells established in long-term culture. The secretion of β_2-microglobulin was relatively uniform when different cell lines were compared, whereas IgG production varied widely. κ- and μ-membrane antigens were modulated by specific antibody; β_2-microglobulin was not modulated. Anti-κ and anti-μ antisera had no effect on the expression of membrane β_2-

microglobulin, nor had anti-β_2-microglobulin antiserum any effect on the expression of κ- and μ-membrane antigens.

Note Added in Proof:—In recent experiments it has been observed that several skin fibroblast cultures from different individuals produce a protein reacting with specific anti-β_2-microglobulin antiserum (Hütteroth, T. H., H. Cleve, S. D. Litwin, and B. S. Danes, unpublished observations).

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