Vol. 140, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1986 Pages 399-405

ANTIBODY TO HUMAN LYMPHOCYTE ACTIN REGULATES IMMUNOGLOBULIN SECRETION BY AN EBV-TRANSFORMED HUMAN B-CELL LINE

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Received August 19, 1986

Antibody against actin isolated from a human EBV-transformed lymphoblastoid B-cell line exerted an inhibitory effect on in vitro IgM secretion by a different lymphoblastoid B-cell line, LA350. This effect was dose dependent showing from 24-40% inhibition at a dilution of 1:100 and 68-80% inhibition at a dilution of 1:50. This effect was noted in the absence of changes in either total cell count or [^{3}H]-thymidine incorporation and was reversed by co-incubation with purified rabbit thymus actin (100 µg/ml) but not bovine serum albumin at the same concentration. These data demonstrate regulation of immunoglobulin synthesis by antibodies against human lymphocyte derived actin in a lymphoblastoid B-cell line.

The presence of actin and actin-like molecules at the cell surface has been reported by several investigators (1-4). We have previously described a stimulatory effect of rabbit antisera and antiactin monoclonal antibodies on murine L-cells (5-7) and have shown significant effects of anti-actin antibodies on proliferation, transmembrane ion fluxes, and membrane phospholipid turnover in both murine and human cell lines (8-11). We now report the effects of an antibody directed against human actin on immunoglobulin production and cell proliferation in a human lymphoblastoid B-cell line (LBL) producing IgM_K immunoglobulin.

MATERIALS AND METHODS

Cell lines: The human B-cell line LA-350 was the kind gift of R.C. Seeger (UCLA Medical Center,Los Angeles, CA). It is an EBV-transformed LBL derived from the peripheral blood of a patient with neuroblastoma prior to

Abbreviations: LBL, lymphoblastoid B-cell line; EBV, Epstein-Barr virus; BSA, bovine serum albumin; HLAct, human lymphocyte actin; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; [^{3}H]-thymidine, tritiated thymidine; CRS, control rabbit serum; RTAct, rabbit thymus actin; CFA, complete Freund's adjuvant; ELISA, enzyme linked immunosorbent assay; SEM, standard error of the mean; SCID, severe combined immunodeficiency.

0006-291X/86 $1.50

chemotherapy. This line spontaneously secretes IgM with predominantly κ-light chains. DV-1 is a spontaneously derived EBV-transformed LBL from a patient with severe combined immunodeficiency who died of a lymphoreticular malignancy (12, and unpublished observations). Details of the production, growth characteristics, and culture conditions of these cell lines have been previously reported (13).

Preparation of Actin and Immunization of Rabbits: Purified human lymphocyte actin (HLAct) was prepared from extracts of DV-1 using a modification of the method of Spudich and Watt reported elsewhere (6). Briefly, DV-1 cells were pooled, washed, and extracted with 1% Triton-X 100 for 1 hr on ice. The monomeric form of actin (G-actin) was isolated from the Triton-X 100 insoluble material. This crude actin was further purified by polymerization to F-actin, precipitation, and resolubilization of G-actin. The final purification involved preparative electrophoresis on 10% polyacrylamide gels, desalting on Bio-Gel PDG-6 and lyophilization and storage at -70°C. The purity of this human lymphocyte actin (HLAct) was analyzed using standard 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and protein staining with Coomassie blue. Rabbit thymus actin (RTAct) was purified in a similar way starting with a commercial preparation of rabbit thymus (Pel-Freez Biologicals Inc., Rogers, Ark.) (6).

Rabbit antisera to this HLAct were produced by immunizing NZW rabbits with 600 µg HLAct in complete Freund's adjuvant (CFA) and boosting with the same dose 2 weeks and 12 weeks later. Antiserum (S24A) was collected 10 days after the last boost. Control rabbit serum (CRS) was produced by immunization with CFA alone.

Immunoreactivity of S24A: Immunoreactivity of S24A was documented on autoradiographs of standard Western blot preparations of purified HLAct. Nitrocellulose strips containing the transferred HLAct were reacted with antisera at a final dilution of 1:500 followed by development with ^{125}I-staphylococcal protein A and autoradiography (6).

Absorption studies were performed by incubation of a 1:50 dilution of S24A with 325 µg/ml of bovine serum albumin (BSA), HLAct, or RTAct followed by ultracentrifugation. Absorbed antisera were then diluted and run on Western blots as described above.

Culture Conditions: Cell cultures were set up in Falcon plastic tubes at a final cell concentration of 5×10^4 cells/ml in RPMI 1640 supplemented with antibiotics and 15% heat inactivated fetal calf serum. In dose response experiments appropriate volumes of either S24A or CRS were added to 1 ml cultures. Reversal experiments were set up in the same manner with the addition of 100 µl of either BSA or purified RTAct at a concentration of 1 mg/ml to each culture tube. After 72hr incubation at 37°C in 5% CO_2, supernatants were collected and held at -20°C until assayed for IgM, and cell pellets were resuspended and counted in cell counting chambers. Proliferation of LA350 was also determined by measuring [^{3}H]-thymidine incorporation after a 48 hour incubation.

IgM Determinations: The IgM content of culture supernatants was determined using a modification of the enzyme linked immunosorbant assay (ELISA) method described by Engvall using 96 well microtiter plates coated with F(ab')$_2$ fragment of goat antihuman IgM (µ- chain specific) (Cooper Scientific, Cochranville. PA.) and developing with alkaline phosphatase conjugated rabbit anti-goat immunoglobulin and 1 mg/ml p-nitrophenylphosphate substrate in a diethanolamine buffer at pH 9.8 (14,15). Optical densities were read using a dual wavelength Dynatech MicroElisa Autoreader, model MR580 at a wavelength of 410 nm. Standard curves were generated using pooled human serum with known IgG, IgA, and IgM content as determined by immunonephelometry and compared to appropriate NIH immunoglobulin standards.

Calculations/Statistics: All experiments were run with either triplicate or quadruplicate cultures set up for each condition. Individual culture supernatants were run in duplicate wells of ELISA plates and IgM was determined by comparison with a standard curve run in triplicate on each plate. When calculated, p-values were derived using Student's 2-tailed

t-test. In Fig.2 the percent inhibition was calculated according to the following equation:

% inhibition of IgM secretion (S) = $[1 - (S)_{24A}/(S)_{CRS}] \times 100$

where:

$(S)_{24A}$ = secretion of IgM in ng/ml/10^5 cells in cultures with S24A

$(S)_{CRS}$ = secretion of IgM as ng/ml/10^5 cells in cultures with CRS

RESULTS

Immunoreactivity of S24A: Lanes 1-3 of the SDS-PAGE demonstrate the purity of the actin preparations used for immunization of rabbits and screening of anti-sera for immune reactivity with HLAct (Fig. 1). The HLAct preparations show at least 90% of the protein contained in a single band at MW_r=43K daltons, corresponding to the commercial alpha actin preparation (lane 2). Lanes 4-9 are autoradiographs demonstrating immunoreactivity of S24A and CRS with HLAct (lanes 4 & 5) and the partial removal of this anti-actin activity by a limited quantity of HLAct and RTAct (lanes 7 & 8 respectively) but not by an equivalent amount of BSA (lane 6). Lane 9 represents the reactivity of a commercially available anti-α-actin antiserum with commercial α-actin. Approximately 80% of the activity was specifically removed by 325 μg of either HLAct or RTAct as estimated by densitometry (data not shown).

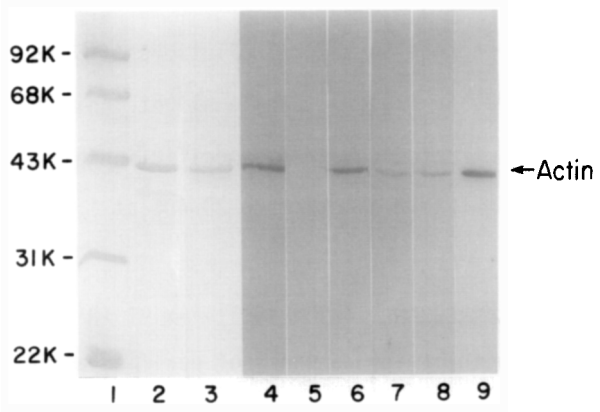

Fig. 1. Lanes 1-3 are 10% SDS-PAGE gels stained for protein with Coomassie blue. Lanes contain as follows: 1) low molecular weight standards, 2) commercially purified α-actin, 3) HLAct isolated and purified as described in "Methods" section above. Lanes 4-9 are autoradiographs of Western blot preparations all using HLAct as the primary antigen and reacted with antisera at a final dilution of 1:500 as follows: 4) S24A, 5) CRS, 6) S24A absorbed with BSA, 7) S24A absorbed with HLAct, 8) S24A absorbed with RTAct, 9) commercial α-actin reacted with commercial antiserum to α-actin. All absorptions were performed as described in "Methods" using 325 μg of the absorbing protein.

Table 1

DOSE RESPONSE FOR S24A INHIBITION OF IGM SYNTHESIS[a]

			Final Serum Dilution	
		Media Control	1:100	1:20
Ex. 1	CRS	105±11	86.5±4	72.5±13
	S24A	-	58.7±3[b]	23.5±3[b]
Ex. 2	CRS	89.3±15	86.5±8	72.5±4
	S24A	-	52±6[b]	23.5±4[b]
Ex. 3	CRS	83±16	76.4±14	72.5±11
	S24A	-	58.7±16[c]	20 ±0[b]

[a]Normalized values represent means ± S.D. for 3 experiments. IgM synthesis expressed in ng/ml/10^5 cells were determined on each of triplicate cultures run in duplicate using the ELISA method outlined in "Materials and Methods".
[b] $p \leq .001$
[c] $p \leq .05$

Effects of S24A on LA-350: Several experiments showed no significant effect of S24A on either viability, cell counts, or [^{3}H]-thymidine incorporation at 24, 48, or 72 hrs of incubation (data not shown). However, significant inhibition of LA350 IgM secretion (expressed as ng/ml/10^5 cells) was detected. The dose response to several dilutions of S24A and CRS in 3 experiments is illustrated in Table 1. While slight inhibition ranging from (3%-28%) was seen in CRS treated cultures, specific inhibition by S24A ranged from a maximum of 70-80% at 1:20 dilution to 20-40% at 1:100 dilutions of antibody.

Specificity of the Response: The specificity of this inhibitory response was tested by reversal with limited amounts of purified actin derived from an independent source (RTAct). Fig. 2 demonstrates significant reduction of the specific inhibitory effect of S24A at final dilutions of 1:100 and 1:20 in 3 separate experiments. For example, in one experiment set up in quadruplicate representative values for IgM secretion were unaffected by the presence of purified RTAct or the BSA control (162 and 189 ng/ml/10^5 cells respectively). When BSA was present at 100 µg/ml, IgM secretion in the presence of 1:100

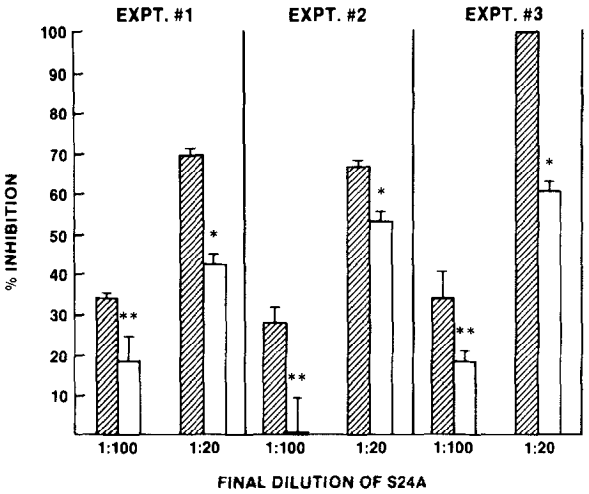

Fig. 2. Reversal of anti-HLAct induced inhibition of IgM secretion using final concentrations of 100µg/ml BSA ▨ or purified RTAct □ at 2 final dilutions of S24A. Results of 3 experiments in which quadruplicate cultures were assayed in duplicate for IgM production (n=8). Values represent mean ± S.E.M. inhibition of IgM sythesis defined by the equation outlined in the "Methods" section.
* $p \leq .001$; ** $p \leq .05$

dilutions of CRS and S24A was 138±24 and 40±5 ng/ml/10^5 cells respectively, representing an inhibition of 71%. The presence of 100 µg/ml of purified RTAct decreased the inhibition to 39% with corresponding IgM synthesis of 132±17 and 80±4 ng/ml/10^5 cells.

DISCUSSION

We have previously reported cAMP dependent effects of anti-actin antibodies on the metabolic activity and proliferation of murine L-cells (16) and the human LBL LA350 (8). In addition we have reported the cell cycle dependent expression of an actin-like molecule on the surface of LA350 using an anti-actin monoclonal antibody (4). In this report we describe the functional effects of a new anti-actin antibody directed against actin purified from a human LBL. In contrast to the significant inhibition of proliferation seen by the anti-RTAct antibody, the anti-HLAct antibody S24A, showed no effect on proliferation of LA350 but did specifically inhibit IgM secretion. Unlike the stimulating activity of anti-µ on resting B-cells (17), activated B-cells have been shown by several groups to demonstrate growth

inhibition upon crosslinking of surface receptors. The crosslinking of surface Ig has been shown by Gordon et al. to inhibit human LBL proliferation (18) and by Hamano et al. (19) to inhibit proliferation while increasing Ig production by a murine hybridoma line. The effects of anti-RTAct on LA350 would appear to resemble the results of Hamano and Gordon whereas; the effect of anti HLAct, S24A, appears to be limited to inhibition of Ig production without altering cell proliferation.

The mechanism of action of anti-actin antibody is not clear but the observed differences between anti-human and anti-rabbit actin on proliferation and immunoglobulin secretion can be explained by several mechanisms: differences in surface binding properties of the two antibodies based on immunoglobulin isotype, degree of glycosylation, or intrinsic binding affinity of each antibody; subtle epitopic differences between RTAct and HLAct might account for the transmission of different activation signals after binding of the respective antibodies to cell membranes; or finally, one might speculate that differences in the intracellular events subsequent to ligand binding at the surface might account for the differences. Events at the translational level could inhibit immunoglobulin secretion without effecting proliferation while events at the transcriptional level would effect both proliferation and secretion.

Although the clinical importance of this inhibitory effect on immunoglobulin secretion is not yet clear, further definition of the mechanisms involved may permit the use of this model system in elucidating effects of anti-actin antibody on humoral immunity in health and disease.

ACKNOWLEDGEMENTS

This work was supported by special grant from Texas Children's Hospital, and Baylor Research Support Grant #G-12311 USPHS Grant RR05425-22.

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