Adult T-Cell Leukemia-Associated Antigen (ATLA): Detection of a Glycoprotein in Cell- and Virus-Free Supernatant

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Z. Naturforsch. **37 c**, 731 – 732 (1982); received June 12, 1982

A glycoprotein of an apparent molecular mass of 46000, gp 46, was enriched by affinity chromatography from the virus- and cell-free culture medium of adult T-cell leukemia virus (ATLV) infected cells. gp 46 was specifically precipitated with sera from patients with adult T-cell leukemia known to react with the adult T-cell leukemia associated antigen (ATLA). Thus, gp 46 is a novel component of the ATLA antigen complex.

Hinuma *et al.* have reported [1] that sera from patients with adult T-cell leukemia (ATL) endemic in south-western Japan [2] react with cytoplasmic antigens of fixed cells of an ATL tumor cell line [3] in an indirect immunofluorescence test. The respective antigen complex was designated adult T-cell leukemia associated antigen (ATLA). In ATL endemic areas about 25% of healthy adults were also found to have ATLA-reacting serum antibodies. Furthermore, ATLA positive cell lines were shown to produce a retrovirus [1, 4]. A similar virus named $HTLV_{CR}$ was isolated independently from a patient with cutaneous T-cell lymphoma in the USA [5]. ATLV could be transmited to other cells by cocultivation with ATLA positive cells accompanied by stabile transformation [6], [7]. Epidemiological, biological and biochemical evidence indicate that ATLV is involved in the etiology of ATL. Thus a characterization of ATLA reactive polypeptides is highly desirable. ATLA antigens could be viral structural polypeptides and their precursors, transforming viral gene products or virally induced cellular polypeptides.

Five polypeptides reacting with ATL-patient sera have already been defined. Posner *et al.* [8] immunoprecipitated p24 and p19 components from an ^{125}I-labelled $HTLV_{CR}$ preparation. Metabolic labelling with ^{35}S-methionine [4, 9] as well as differential iodination of particles [10] indicated that p24 is a viral core polypeptide. In addition, Yamamoto and Hinuma [9] detected four ^{35}S-methionine-labelled patient sera in HTLV producing MT-2 cells [6]. The

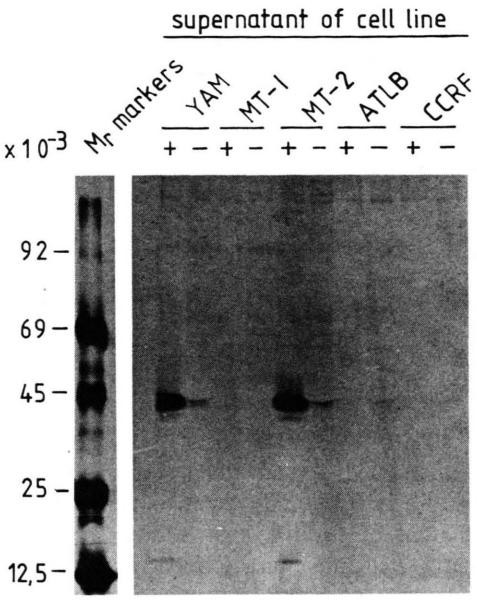

Fig. 1. PAGE analysis of immunoprecipitated soluble ATLA glycoprotein. The human lymphoblastoid cell lines described in Table I were maintained in RPMI 1640 culture medium supplemented with 10% heat inactivated (56 °C, 30′) fetal bovine serum (FCS; Gibco-Europe, Karlsruhe). 5 ml of a cell suspension (8×10^5 cells per ml) were labelled for 15 – 16 h with 50 – 100 µCi/ml of ^{35}S-cystein (Amersham, Braunschweig) in medium without cystein and substituted with dialysed FCS. The cell and particle free culture supernatant was obtained by centrifugation at 4 °C (2000 rpm, 5′; 4000 rpm, 10′; Beckman rotor SW41 30000 rpm, 53′; respectively). Glycoproteins were absorbed from each supernatant to concanavalin A-bound Sepharose (1 ml settled bed of Con A-Sepharose, Pharmacia, Freiburg) for two hours at 4 °C. The Con-A-Sepharose was then poured into a column, washed with 10 ml of PBS (phosphate buffered, pH 7.2, saline) and eluted specifically with 2 ml of 0.3 M α-methyl-mannoside (Fluka, Buchs, Switzerland). For immunoprecipitation five ATLA reactive sera from Japanese ATL patients and 5 ATLA non-reactive sera (3 japanese, 2 german) were employed. The patient serum used in this experiment had an ATLA antibody titer of 1 : 1280 in the indirect immunofluorescence test. 500 µl of the Con-A eluate were incubated with 5 µl of serum for 10 hours at 4 °C. Immune complexes were harvested with 10 mg of protein-A bound Sepharose (Pharmacia, Freiburg), washed as described [9] and separated on a 9 – 16% acrylamide gradient PAGE under reducing conditions. Gels were treated for fluorography [13]. (+) precipitation with ATLA reactive patient serum; (−) ATLA non-reactive patient serum. ^{14}C labelled marker proteins were run on the same gel. (Phosphorylase b, 92000; bovine serum albumine, 68000; ovalbumine 45000; chymotrypsinogen, 25000; cytochrome c, 12500).

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0341-0382/82/0700-0731 $ 01.30/0

Table I. Characteristics of human lymphoblastoid cell lines.

Cell line		Immunofluorescence[a]		Reference
Designation	Surface[b] markers	ATLA %	EBNA[d] %[e]	
CCRF-CEM	T	0	0	Foly *et al.* [11]
MT-1	T	1–3	0	Miyoshi *et al.* [3]
ATLB 2–23	B	25[f]	>90	Yamamoto *et al.* [12]
MT-2	T	>95	0	Miyoshi *et al.* [6]
YAM	T	>95	0	Yamamoto *et al.* [7]

[a] Indirect immunofluorescence test on methanol fixed cells.
[b] Taken from the reference.
[c] Adult T-cell leukemia associated antigen detected as cytoplasmic and membrane fluorescence with sera from ATL patients.
[d] Epstein-Barr Virus nuclear antigen detected as brilliant nuclear fluorescence with human reference sera.
[e] Number of positive cells in percent.
[f] Detected with a monoclonal mouse antibody to the ATLA component p28 (Tanaka *et al.*, in preparation).

aim of this study was to identify possible glycoproteins of the ATLA system.

^{35}S-cystein labelled glycoproteins were enriched by concanavalin A from cell- and particle-free culture supernatant of four ATLA-positive and one ATLA-negative cell line characterized in Table I. A glycoprotein with an apparent molecular mass of 46 000 was specifically precipitable with a panel of 5 sera from ATLV patients from supernatants of YAM and MT-2 cells. A representative precipitation pattern is shown in Fig. 1. Interestingly, the two lines in which only a minor fraction of cells expressed ATLA, MT-1 and ATLB 2–23, were negative as were the controls CCRF-CEM. Thus, the concentration of gp 46 appears to correlate with ATLA expression. EBV related antigens were not detected in our assay by any of the 10 sera.

From these results we conclude that gp 46 is a soluble, additional polypeptide with ATLA reactivity. The possible relation of gp 46 to viral or cellular ATLA components is under investigation.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Hu 244/6 and Kl 417/2). N.Y. received a Yamgiwa-Yoshida Memorial International Cancer Study Grant.

Note added in proof: Further results indicated that gp 46 is a viral gene product.

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Verantwortlich für den Inhalt: A. KLEMM
Satz und Druck: Konrad Triltsch, Würzburg