α_2HS GLYCOPROTEIN BINDS TO LYMPHOCYTES TRANSFORMED BY EPSTEIN–BARR VIRUS

J. G. LEWIS, P. S. CROSIER* and C. M. ANDRÉ

Departments of Clinical Biochemistry and **Medicine, Christchurch Clinical School of Medicine, Christchurch Hospital, Christchurch, New Zealand*

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1. Introduction

The serum protein α_2HS glycoprotein acts as an opsonin during bacterial phagocytosis by neutrophils [1] and enhances macrophage phagocytic function [2]. In addition α_2HS glycoprotein binds to DNA [3] and promotes the endocytosis of radio-labelled DNA by mouse macrophages [4]. DNA–protein complexes are present on the surface membrane of Ehrlich ascites tumour cells [5]. We therefore wondered whether α_2HS glycoprotein could bind to lymphocytes transformed by Epstein–Barr virus (EBV) a DNA virus which is implicated as an oncogenic agent [6]. This could presuppose a role for α_2HS glycoprotein as a soluble mediator enhancing cell-mediated host defense mechanism against EBV-transformed cells. The binding of α_2HS glycoprotein to EBV-transformed lymphocytes was studied by indirect immunofluorescence as well as directly by the use of ^{125}I-labelled α_2HS glycoprotein. We conclude α_2HS glycoprotein binds appreciably to EBV-transformed but not to normal autologous non-transformed lymphocytes, $K_d = 5.0 \times 10^{-7}$ M with 0.5×10^6 sites/cell.

2. Materials and methods

α_2HS glycoprotein was isolated from serum [4] and stored at −20°C (1.6 mg/ml in phosphate-buffered saline, PBS).

Peripheral blood lymphocytes (PBL) were separated by density-gradient centrifugation and transformed [7] with EBV-containing culture supernatants derived from the B95-8 cell line. The EBV-transformed cell line was free from both *Mycoplasma* and bacterial infection. Both transformed and normal autologous lymphocytes were cultured in Hepes-buffered RPMI supplemented with 15% fetal calf serum (FCS, Gibco Batch 182), 2 mM L-glutamine and maintained in 95% air/5% CO_2 at 37°C.

For indirect immunofluorescence eosin-viable transformed cells (10^7) were washed 3 × with Hepes-buffered RPMI supplemented with 15% FCS (Gibco, Batch 182), 2 mM L-glutamine, 0.02% NaN_3 (RPMI/FCS) and incubated 60 min at 22°C with an equal volume of purified α_2HS glycoprotein solution (1.6 mg/ml in PBS). The cells were rewashed 3 × with RPMI/FCS and incubated, 60 min at 22°C, with either 1:10 dilution of rabbit anti-α_2HS glycoprotein (Behringwerke) in RPMI/FCS or 1:10 dilution of non-immune rabbit serum in RPMI/FCS as control. Cells were further washed and incubated, 60 min at 22°C, with 1:10 dilution of sheep anti-rabbit-FITC serum (Wellcome) in RPMI/FCS. Following final washing the cells were viewed under UV light.

^{125}I-Labelled α_2HS glycoprotein [8] was used to further analyse the binding to transformed lymphocytes. Aliquots (50 μl) of cell suspension in RPMI were added to 50 μl ^{125}I-α_2HS glycoprotein (1 μg containing 10^6 cpm/μg in PBS) in siliconised glass tubes for incubation at 4°C. Following incubation, ice-cold RPMI (300 μl) was dispensed into each tube and the contents layered onto a cold Ficoll–hypaque density gradient, specific gravity 1.040, and centrifuged at 5000 rev./min for 30 min. The supernatant was aspirated and the pelleted cells resuspended in Ca^{2+},Mg^{2+}-free Hanks balanced salt solution (HBSS) containing 0.1% trypsin, 0.2% EDTA (100 μl). The cell suspension was transferred to tubes for counting the cell bound ^{125}I-α_2HS glycoprotein. Blanks were also performed in the absence of cells to measure non-specific binding. Binding was expressed as cpm.

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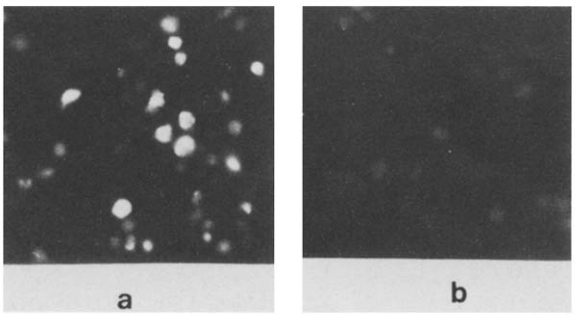


Fig.1. Binding of α_2HS glycoprotein to EBV-transformed lymphocytes by indirect immunofluorescence. Cells were incubated with an equal volume of α_2HS glycoprotein solution, then with either rabbit anti-α_2HS glycoprotein (a), or non-immune rabbit serum as control (b), and finally with fluorescein-labelled sheep antibody to rabbit serum.

Table 1
Binding of α_2HS glycoprotein to EBV-transformed and normal non-transformed autologous lymphocytes by indirect immunofluorescence

Cell type	Fluorescence
EBV-transformed lymphocytes	+
Normal lymphocytes (t = 0)	–
Normal lymphocytes (t = 1 day)	–
Normal lymphocytes (t = 8 days)	–

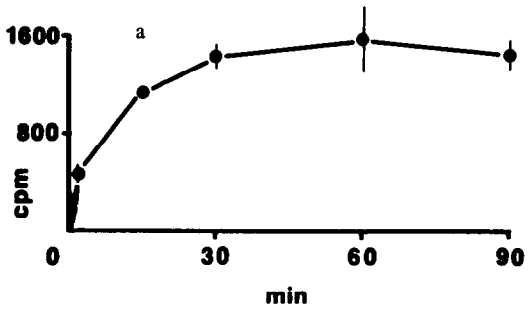
Indirect immunofluorescence of viable EBV-transformed lymphocytes (10^7) and viable normal autologous lymphocytes (10^7) stained immediately t = 0; and following 1 or 8 days culture in Hepes-buffered RPMI supplemented with 15% FCS and 2 mM L-glutamine. PBL from the original donor served as the normal non-transformed autologous cell control

3. Results and discussion

There was bright fluorescence in the transformed cells incubated with rabbit anti-α_2HS glycoprotein serum with virtually no fluorescence in either the control (non-immune rabbit serum) (fig.1) or in normal autologous lymphocytes, whether stained immediately or following 1 or 8 days in culture (table 1). The fluorescence observed in the transformed compared to normal autologous lymphocytes is unlikely to be due simply to the B cell selectivity of the virus as normal B cells cultured in this medium show increased proliferation compared to T cells (P. S. C., unpublished). Specificity of immunofluorescence was confirmed by the use of α_2HS glycoprotein antiserum absorbed with α_2HS glycoprotein. The absorbed antiserum failed to react with normal human serum by immunodiffusion and did not produce fluorescence with the transformed cells.

With ^{125}I-α_2HS glycoprotein binding equilibrium was attained after 30 min and was dependent on cell concentration (fig.2a,b). The dose dependence of α_2HS glycoprotein is shown (fig.3). Scatchard plot analysis [9] (inset fig.3), revealed $K_d = 5.0 \times 10^{-7}$ M with 0.5×10^6 sites/cell. The affinity is similar to that of α_2HS glycoprotein for calf thymus DNA, $K_d = 9.0 \times 10^{-7}$ M (unpublished).

We speculated that DNA might be implicated in binding of α_2HS glycoprotein to transformed cells. However, pre-treatment of the EBV-transformed cells with DNase (0.5 mg/ml in HBSS) 2000 Kunits units/



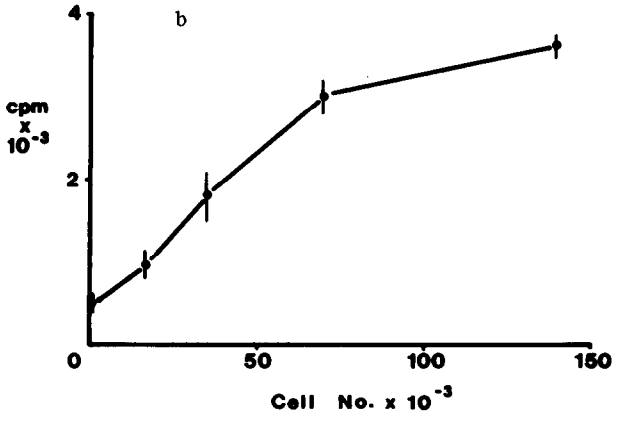


Fig.2. Binding of ^{125}I-α_2HS glycoprotein to EBV transformed lymphocytes. Dependence on incubation time at 4°C (10^5 cells) after blank value correction (a), mean ± SEM, n = 4; and cell concentration (expressed as cell no./50 μl aliquot) for 60 min incubation at 4°C (b), mean ± SEM, n = 5.

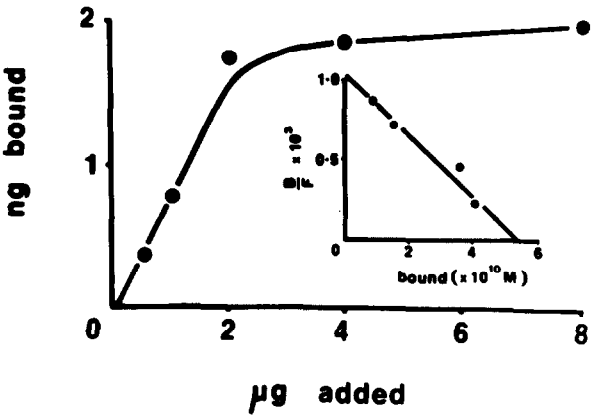


Fig.3. Concentration dependence of ^{125}I-α_2HS glycoprotein on binding after blank value correction. Cells (0.6×10^5) incubated for 60 min at 4°C with varying amounts of ^{125}I-α_2HS glycoprotein containing 0.45×10^6 cpm/µg. The Scatchard plot (inset) revealed $K_d = 5.0 \times 10^{-7}$ M with 0.5×10^6 sites/cell. Each point is the mean of triplicate determinations.

mg, did not significantly diminish fluorescence or binding of ^{125}I-α_2HS glycoprotein. Although we have demonstrated that α_2HS glycoprotein, at physiological concentration (0.8 mg/ml), binds appreciably to EBV-transformed lymphocytes the nature of the receptor remains obscure. Preliminary experiments indicate both fluorescence and binding of ^{125}I-α_2HS glycoprotein can be partially blocked by pre-treatment of the transformed cells with RNase (2 mg/ml in HBBS) 80 Kunitz units/mg or pronase (2 mg/ml in HBBS), although direct confirmation awaits isolation of the α_2HS glycoprotein receptor. Interestingly, B cells harbouring the EBV genome can synthesise novel RNAs which may be complexed with protein [10,11] and have been located in the cytoplasm. Whether such complexes become associated with the outer membrane is unknown.

α_2HS glycoprotein acts as an opsonin during bacterial phagocytosis by human neutrophils [1] and enhances latex phagocytosis by macrophages [2,3]. These findings of the binding of α_2HS glycoprotein to EBV-transformed lymphocytes necessitate investigations as to whether α_2HS glycoprotein can stimulate macrophage-mediated phagocytosis or enhance T cell-mediated cytolysis of these transformed cell lines. In addition, the binding of α_2HS glycoprotein to other EBV-carrying and EBV-negative cell lines merits investigation.

Acknowledgements

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References

[1] Van Oss, C. J., Gillman, C. F., Bronson, P. M. and Border, J. R. (1974) Immunol. Commun. 3, 329–335.
[2] Lewis, J. G. and André, C. M. (1981) Immunology 42, 481–487.
[3] Lewis, J. G. and André, C. M. (1978) FEBS Lett. 92, 211–213.
[4] Lewis, J. G. and André, C. M. (1980) Immunology 39, 317–322.
[5] Schell, P. L. (1978) Z. Naturforsch 33c, 96–104.
[6] Epstein, M. A. and Archong, B. G. (1979) in: The Epstein–Barr Virus (Epstein, M. A. and Archong, B. G. eds) pp. 1–22, Springer, Berlin, New York.
[7] Sugden, B. and Mark, W. (1977) J. Virol. 23, 503–508.
[8] Hunter, W. M. (1974) Brit. Med. Bull. 30, 18–23.
[9] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–672.
[10] Lerner, M. R., Andrews, N. C., Miller, G. and Steitz, J. A. (1981) Proc. Natl. Acad. Sci. USA 78, 805–809.
[11] Van Santen, V., Cheung, A. and Kieff, E. (1981) Proc. Natl. Acad. Sci. USA 78, 1930–1934.