ELSEVIER

Biochimica et Biophysica Acta 1284 (1996) 1–3

BBA Biochimica et Biophysica Acta

Rapid report

Both the H13 gene product and 4F2 antigen are involved in the induction of system y^+ cationic amino-acid transport following activation of human peripheral blood mononuclear cells (PBM)

Sarah Chen, David Meredith, C.A. Richard Boyd *

Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK

Received 22 April 1996; revised 31 May 1996; accepted 4 July 1996

Abstract

Prior transfection with antisense oligonucleotides to the H13 and 4F2 hc genes, singly or in combination, was found to inhibit phytohaemagglutinin-induced activation of cationic amino-acid transport system y^+ in human peripheral blood mononuclear cells (mostly circulating lymphocytes). These effects on system y^+ function or expression mean that 4F2 hc cannot only be the molecular basis of system y^+L (Fei, Y.-J., Prasad, P.D., Leibach, F.H. and Ganapathy, V. (1995) Biochemistry 34, 8744–8751).

Keywords: Cationic amino acid transport; Amino acid transport; Peripheral blood mononuclear cell; H13 gene; 4F2 gene; Antisense oligonucleotide; Oligonucleotide; (Human)

Two distinct systems have been described for cationic amino-acid transport in mammalian cells. System y^+, a low-affinity, high-capacity system [2] and system y^+L a high-affinity, low-capacity system [3]. These systems are in addition to system $b^{0,+}$ and $B^{0,+}$ which have broad specificity but which only contribute significantly in specific tissues. Recent studies have shown that the molecular basis of system y^+ transport in murine cells is a family of proteins encoded by the *mCAT* (murine cationic amino-acid transporter) genes *mCAT*-1 and *mCAT*-2 [4–6]. The structure of these proteins, mCAT-1, mCAT-2A and mCAT-2B, is highly similar and predicted to consist of multiple (12–14) membrane spanning domains. This is characteristic of most known transporters. It has been suggested that the 4F2 hc antigen may be the molecular basis of system y^+L [1]. So far, only the heavy chain of the 4F2 antigen (4F2 hc) has been cloned and unlike the mCAT proteins, the 4F2 hc is predicted to have only a single transmembrane domain.

Cationic amino-acid transport through both systems occurs in human PBM and is increased following activation of the cells with the mitogen phytohaemagglutinin (PHA), but to different extents and with different time-courses [7]. The H13 gene, a human gene homologous to *mCAT*-1 (87% amino-acid homology), has been cloned from a cDNA library derived from a human T cell line [8]. The protein encoded by this gene is structurally similar to the mCAT proteins (containing multiple membrane spanning domains) and is likely to be the molecular basis of system y^+ in lymphocytes. The 4F2 antigen is not expressed at significant levels on resting lymphocytes, but its expression is induced following lectin or allogenic stimulation. It is one of the earliest known T cell activation antigens.

The molecular basis of the induced cationic amino-acid transport stems was investigated by hybrid depletion using antisense oligonucleotides designed to the H13 and 4F2 genes. PBM were separated as previously described [7] from buffy coat preparations of human peripheral blood obtained from healthy donors.

Sense and antisense oligonucleotides were synthesised for each gene and an identical strategy was used in both cases. The oligonucleotides were 18mer in length and designed in such a way that the position of the initiation codon was in the middle of the oligonucleotides. The sequence of the H13 sense and antisense oligonucleotides was 5′-ACAGCAACATGGGGTGCA-3′ and 5′-TGCACCCCATGTTGCTGT-3′, respectively. For the 4F2 gene these were 5′-CAGGCACCATGAGCCAGG-3′ and 5′-CCTGGCTCATGGTGCCTG-3′, respectively. These sequences

* Corresponding author. Fax: +44 1865 272420; e-mail: carboyd@vax.ox.ac.uk.

PII S0005-2736(96)00125-3

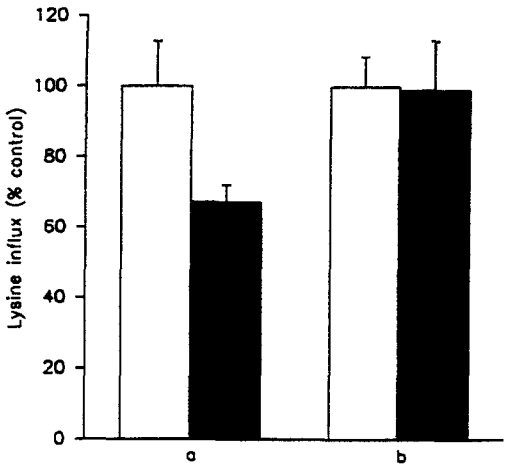

Fig. 1. The effect on system y^+ (a) and system y^+ L (b) of transfecting H13 sense (open bars) and antisense (filled bars) oligonucleotides. Following transfection, the cells were activated with pHA (10 μg/ml) for 24 h then washed thoroughly with Kreb's Ringer solution before initial rates of lysine uptake were measured. The graph shows the mean ± S.E.M. from 11 experiments.

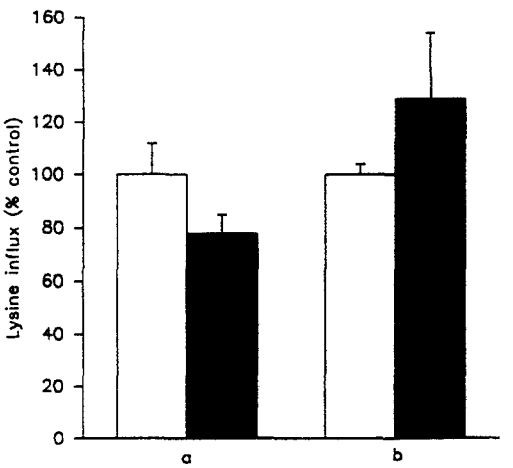

Fig. 3. The effect on system y^+ (a) and system y^+ L (b) of simultaneously transfecting both H13 and 4F2 hc sense (open bars) and antisense (filled bars) oligonucleotides. Following transfection, the cells were activated with pHA (10 μg/ml) for 24 h then washed thoroughly with Kreb's Ringer solution before initial rates of lysine uptake were measured. The graph shows the mean ± S.E.M. from 15 experiments.

were found to be unique when compared with all other human sequences present in the sequence databases.

Lipofectin reagent (GIBCO BRL), a liposome formulation, was used for delivery of the oligonucleotides. The protocol used was based on the method described by Chiang et al. [9]. Lipofectin (4 μl/ million (10^6) cells) and the oligonucleotide (6 μg/ million cells) were preincubated in 1 ml serum free RPMI medium (ICN FLOW) at room temperature for 15–20 min. PBM in an equal volume of serum free RPMI medium were added to the mixture and incubated in 5% CO_2 at 37°C for 4 h. PBM were then centrifuged and resuspended at a cell density of 2 million/ml in growth medium containing 10 ng/ml PHA (Sigma) and 6 μg/ml oligonucleotide. Following 24 h of culture, the cells were washed thoroughly using Kreb's Ringer solution, before transport of 2 μM lysine through systems y^+ and y^+L was measured under initial rate conditions as previously described [7]. In brief the former system was determined in the presence of 0.1 mM leucine, and the latter as the difference between the total transport rate and that in the presence of the neutral amino acid. Previous work [7] showed that the rates so measured were similar to the direct estimate of y^+L transport as measured by selective inhibition of y^+ with *N*-ethylmaleimide.

Preliminary experiments carried out showed that there were no non-specific effects of transfecting sense oligonucleotides into PBM since transport in sense transfected

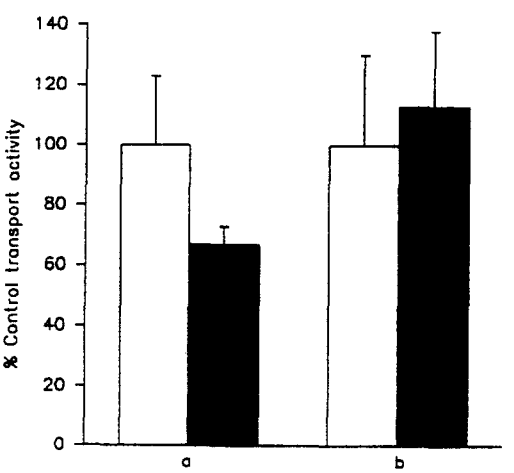

Fig. 2. The effect on system y^+ (a) and system y^+ L (b) of transfecting 4F2 hc sense (open bars) and antisense (filled bars) oligonucleotides. Following transfection, the cells were activated with pHA (10 μg/ml) for 24 h then washed thoroughly with Kreb's Ringer solution before initial rates of lysine uptake were measured. The graph shows the mean ± S.E.M. from four experiments.

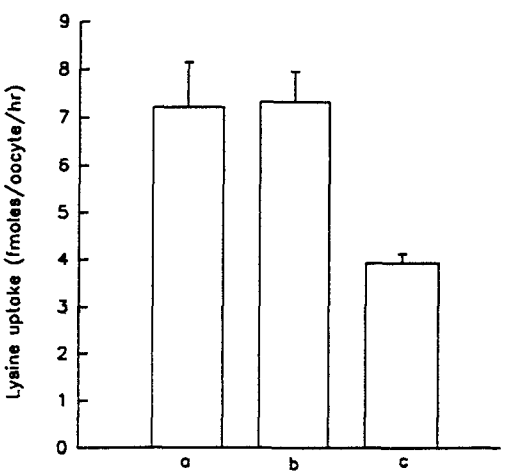

Fig. 4. Uptake of 28 nM lysine into *Xenopus laevis* oocytes (mCAT-1 RNA injected (a), mCAT-1 RNA and sense oligonucleotide (b) and mCAT-1 RNA and antisense oligonucleotide (c)). Lysine uptakes were measured in the presence of 500 μM L-leucine 5 days after injection. 37.5 ng of RNA and a 10-fold excess of oligonucleotide was injected into each oocyte. The graph shows the mean ± S.E.M. uptake of 5–7 oocytes.

cells was not significantly different to untransfected cells ($P = 0.9$, (t test) $n = 10$). Therefore, in subsequent experiments transport by sense transfected cells was used as a control.

Influx of 2 μM lysine in H13 oligonucleotide transfected cells (as % sense control, mean ± S.E.M.) through system y^+ and system y^+L was 67% ± 5 ($P = 0.001$, $n = 11$) and 99% ± 14, respectively (Fig. 1). When PBM were transfected with 4F2 hc oligonucleotides the resulting effect on cationic amino-acid transport was unexpected and similar to that observed with the H13 oligonucleotides; there was no significant reduction in system y^+L but a significant reduction in system y^+ transport. Influx of 2 μM lysine (as % sense control, mean ± S.E.M.) through system y^+ and y^+L was 67% ± 6 ($P \leq 0.01$, $n = 4$) and 113% ± 25, respectively (Fig. 2). When both H13 and 4F2 hc oligonucleotides were transfected simultaneously (Fig. 3), a significant reduction in system y^+ was observed (78% ± 7, $P = 0.02$, $n = 15$). The effect on system y^+L was variable and overall not significant (129% ± 25, $n = 15$).

The specificity of oligonucleotide design was confirmed by using an identical strategy to synthesise sense and antisense oligonucleotides to mCAT-1. mCAT-1 cDNA (a gift from Dr. J.M. Cunningham) was used to prepare RNA which was co-injected with sense or antisense oligonucleotides into *Xenopus laevis* oocytes [4]. Antisense oligonucleotide injected oocytes had significantly reduced transport ($P = 0.005$) through system y^+ when compared to sense injected oocytes; the latter were not different from cRNA only injected oocytes (Fig. 4).

In human PBM the H13 gene product, referred to as the human retroviral receptor or hCAT-1, is most likely to be the basis of system y^+. Indeed PBM transfected with antisense oligonucleotides to this gene showed significant reduction in system y^+ transport (67% of sense control) compared to cells transfected with the sense oligonucleotide. In these experiments, system y^+L served as a useful internal control since no difference in transport activity was observed in sense and antisense transfected cells.

It has been suggested that 4F2 hc is the molecular basis of system y^+L [1]. However, when antisense oligonucleotides to 4F2 hc gene were transfected into PBM, there was a very variable effect on system y^+L, with the overall result from four separate experiments of no change in activity in the antisense transfected cells as compared to the sense and untransfected controls. (Possible explanations for this negative result include the small absolute increase in y^+L activity following activation [7] and the greater technical difficulty in accurate determination of y^+L since it is detected by difference.) In contrast, a consistent reduction in transport through system y^+ was observed and this was to a similar extent to that observed in PBM transfected with H13 antisense oligonucleotides. A possible role of the 4F2 antigen in y^+L activity is supported by the recent paper by Fei et al. [1], where y^+L-like transport is induced in oocytes injected with mRNA prepared from a human choriocarcinoma cell line (JAR) which expresses high levels of the 4F2 antigen. This activity was abolished when an antisense oligonucleotide to 4F2 hc similar to that used in the experiments described above was coinjected into *Xenopus* oocytes with the total mRNA from the JAR cells.

It is unlikely that the antisense reduction of system y^+ with either 4F2 hc or H13 reported here is due solely to an inhibition of cell activation since injection of mCAT-1 (Fig. 4) and 4F2 hc cRNA (Bertran et al. [10], Wells et al. [11]) results in y^+ activity in *Xenopus* oocytes.

Our findings show that the product of the 4F2 hc gene must have roles additional to that proposed by Fei et al. [1]. It is intriguing that the single transmembrane domain protein appears to be involved in the function of two distinct amino-acid transport systems.

The authors would like to thank the Birmingham Regional Blood Transfusion Centre for providing buffy coat preparations, Dr. N.J. Proudfoot (Sir William Dunn School of Pathology, University of Oxford) for synthesis of oligonucleotides and Dr. C.F. Newland (Institute of Molecular Medicine, Oxford) for providing *Xenopus* oocytes and injection facilities. S.C. was in receipt of a Medical Research Council Studentship.

References

[1] Fei, Y.-J., Prasad, P.D., Leibach, F.H. and Ganapathy, V. (1995) Biochemistry 34, 8744–8751.
[2] Christensen, H.N. (1964) Proc. Natl. Acad. Sci. USA 51, 337–344.
[3] Deves, R., Chavez, P. and Boyd, C.A.R. (1992) J. Physiol. 454, 491–501.
[4] Kim, J.W., Closs, E.I., Albritton, L.M. and Cunningham, J.M. (1991) Nature 352, 725–728.
[5] Wang, H., Kavanaugh, M.P., North, R.A. and Kabat, D. (1991) Nature 352, 729–731.
[6] Closs, E.I., Albritton, L.M., Kim, J.W. and Cunningham, J.M. (1993) J. Biol. Chem. 268, 7538–7544.
[7] Boyd, C.A.R. and Crawford, D.H. (1992) Eur. J. Physiol. 422, 87–89.
[8] Yoshimoto, T., Yoshimoto, E. and Meruelo, D. (1991) Virology 185, 10–17.
[9] Chiang, M.Y., Chan, H., Zounes, M.A., Freier, S.M., Lima, W.F. and Bennett, C.F. (1991) J. Biol. Chem. 266, 18162–18171.
[10] Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kuhn, L.C., Palacin, M. and Murer, H. (1992) Proc. Natl. Sci. USA 89, 5606–5610.
[11] Wells, R.G., Lee, W.S., Kanai, Y., Leiden, J.M. and Hediger, M.A. (1992) J. Biol. Chem. 267, 15285–15288.