Expression of human α_1-antitrypsin using a recombinant adenovirus vector

P. Gilardi[1], M. Courtney[2,*], A. Pavirani[2] and M. Perricaudet[1]

[1] *Institut Gustave Roussy, 39 Rue Camille Desmoulins, 94800 Villejuif, France* and [2] *Transgene SA, 11 Rue de Molsheim, 67082 Strasbourg Cedex, France*

Received 20 March 1990; revised version received 27 April 1990

In order to improve the in vivo delivery of human proteins destined for replacement therapy, a recombinant adenovirus containing the cDNA sequence encoding human α_1-antitrypsin has been constructed and shown to direct expression of the protein in the supernatant of human cell lines.

Antiprotease, Serine protease, α_1-Antitrypsin, Gene targeting; Drug delivery, in vivo, Recombinant adenovirus

1. INTRODUCTION

α_1-Antitrypsin (α_1AT) is a serum antiprotease which is mainly produced by hepatocytes and mononuclear phagocytes and whose major physiological function is to inhibit neutrophil elastase (NE) activity in the lung [1]. NE is a serine protease capable of degrading proteins of the alveolar interstitium, the result of this action being the destruction of the lung parenchyma [2]. Severe hereditary deficiency of α_1AT is associated with low plasma and lung levels of α_1AT and predisposes affected individuals to pulmonary emphysema by impairing the protease/antiprotease balance in the lower respiratory tract [3,4].

α_1AT replacement is currently being assessed in the therapy of hereditary emphysema [5]. Large quantities of recombinant unglycosylated α_1AT have been made available by production in *E. coli* or yeast but the absence of sugar side chains results in a shorter plasma half-life [6–8].

To circumvent this problem the product could be delivered directly to the site where the pathology of the deficiency is manifest, i.e. the alveolar epithelium. Aerosol administration of recombinant α_1AT has been shown to be effective in α_1AT-deficient patients [8,9]. Another possible approach is to implant in the lung autologous cells secreting 'normal' α_1AT or to deliver the correct α_1AT gene in situ via, for instance, a recombinant viral vehicle infecting the lung epithelial cells. Both approaches rely on an efficient vector suited for high level and stable expression of α_1AT in exogenous cells.

Retroviral vectors have recently been used to produce glycosylated recombinant α_1AT [10]. Transformed fibroblasts have been implanted in the peritoneal cavity of nude mice and human α_1AT secreted by these cells has been detected in plasma and in the epithelial fluid of the lungs [11]. Although confirming the feasibility of the strategy, these experiments, however, demonstrated the need for improvement in the technology since the amounts of α_1AT produced by the retrovirus-infected cells were low.

2. EXPERIMENTAL

2.1 *Construction of the expression plasmid MLP-α_1AT*

Plasmid MLP-α_1AT contains the following sequences inserted between the *Eco*RI and *Bam*HI restriction sites of pBR322. (i) The 455 base pairs of the left end of the human adenovirus (Ad) type 5 genome (nucleotides 1–455). This fragment carries the Inverted Terminal Repeat, the encapsidation signal sequences and the enhancer of the E1A promoter. (ii) The major late promoter (MLP) of the human Ad type 2 (nucleotides 5780–6038), followed by an almost complete copy of the tripartite leader cDNA sequence (nucleotides 6039–6079; 7100–7171; 9634–9713) (iii) The complete human α_1AT cDNA and (iv) the polyadenylation signal from the SV40 A gene (nucleotides 2666–2533).

2.2 *Construction of recombinant adenovirus*

The plasmid MLP-α_1AT was digested with *Pst*I and *Acc*I within the pBR322 sequence, and the excised fragment was cloned into the unique *Cla*I site (map units 2.6) of Ad type 5, thus replacing a portion of the viral E1A gene (Fig. 1). To obtain an acceptable DNA size for encapsidation, it was necessary to use an Ad deleted in the E3 region (d1327), which is not essential for the growth of the virus in vitro or in vivo. After ligation, the DNA was used to transfect Ad type 5-transformed human 293 cells [12], using calcium phosphate precipitation [13]. A recombinant adenovirus-α_1AT (Ad-α_1AT) was isolated after 7 days, as shown by DNA analysis.

Correspondence address: M. Perricaudet, Génétique des Virus Oncogènes, Institut Gustave Roussy/PR 2, 39 Rue Camille Desmoulins, 94805 Villejuif Cedex, France

* *Present address:* Research Department, Delta Biotechnology Ltd, Castle Court, Castle Boulevard, Nottingham NG7 1FD, UK

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

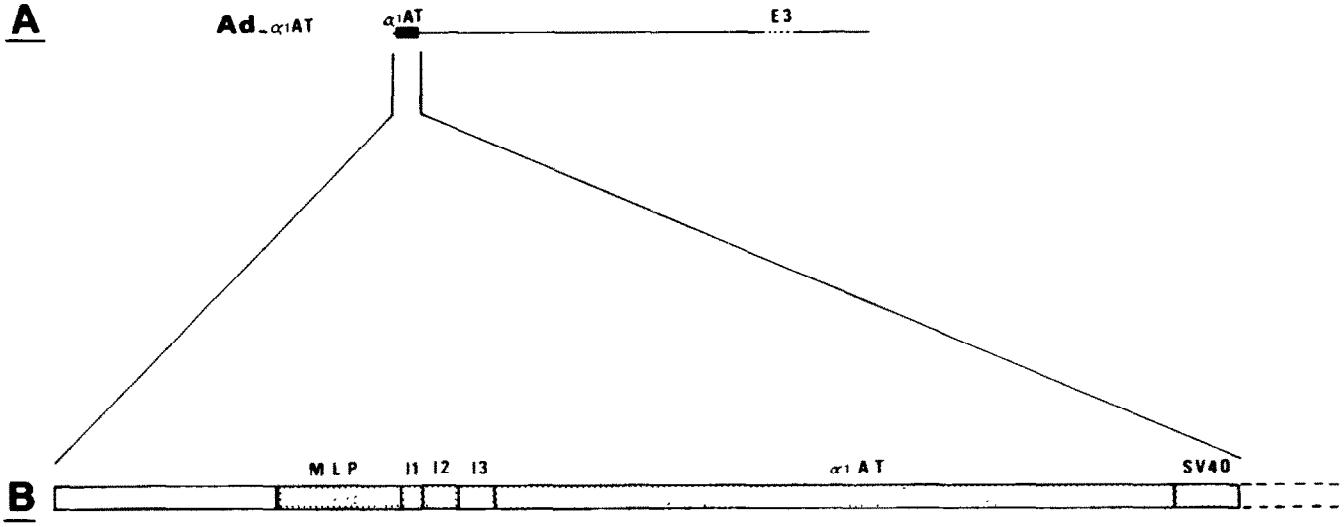

Fig. 1. (A) Structure of the recombinant adenovirus (Ad-α_1AT): the Ad5 d1327 derivative, which has the E3 region deleted, was used as vector for the chimeric α_1AT transcription unit. (B) Enlarged is the left end of the recombinant genome containing the chimeric α_1AT transcription unit: sequences corresponding to the major late promoter (MLP) and to the tripartite leader (11, 12, 13) have been used to drive the transcription of the α_1AT cDNA and the SV40 mRNA processing signals.

2.3. *α_1AT expression*

α_1AT expression was monitored by ELISA essentially as described in [14]. Goat anti-human α_1AT (Cappel, Melvern, PA, USA) was used as the primary and secondary antibody, the latter being tagged with peroxidase.

3. RESULTS AND DISCUSSION

Transient expression of α_1AT was tested in CHO cells after transfection with plasmid MLP-α_1AT. At 25 h post-transfection, 2.5 μg/ml of α_1AT were found in the supernatant (not shown). This demonstrated that the MLP directs the transcription of the α_1AT cDNA into an mRNA that is efficiently translated.

Expression levels of α_1AT synthesized after Ad-α_1AT infection of 293 and HeLa cells are presented in Table I. Infection by the recombinant adenovirus at a multiplicity of 10 or 100 p.f.u. per cell led to accumulation of α_1AT in the medium. After 6 days of infection with 100 p.f.u. of HeLa cells 60 μg/ml of α_1AT were detected. The results show that Ad can be used in vitro to express high levels of human α_1AT. Amounts are higher for HeLa cells (which do not complement the E1A defect) than for 293 cells where the recombinant virus can grow efficiently. This observation can be explained by the fact that in HeLa cells the cellular machinery can transcribe the viral genes more efficiently as it is less compromised by the growth of the virus.

Table I

Time course of extracellular α_1AT production

Day	293 cells		HeLa cells	
	10 p.f.u.	100 p.f.u.	10 p.f.u.	100 p.f.u.
1	1.933	3.937	0	0.625
2	3.750	5.400	0.325	4.375
3	4.250	5.050	3.800	41.250
6	4.250	5.500	31.250	60.000

The cumulative amounts of human α_1-antitrypsin (expressed in μg/ml) produced after infection of 1×10^7 293 and HeLa cells with 10 or 100 p.f.u. of Ad-α_1AT virus

4. CONCLUSION AND PROSPECTS

With the goal of improving the expression of recombinant glycosylated α_1AT we have explored the use of an Ad vector. Ad vectors have proved useful for achieving high-level expression of a variety of foreign genes in different cell types [15–19]. Ad infection in humans is benign and no neoplastic transformation has been associated. Here we report the construction of a recombinant Ad bearing the cDNA of the human α_1AT under the control of the viral major late promoter. Since the E1A region is replaced, this vector is replication-defective unless propagated in human 293 cells which complement the E1A defect, but can infect a variety of cell types where stable genomic integration of the viral sequences can occur.

Our results argue in favor of the possibility of a recombinant adenovirus vector being used to correct in vivo α_1AT deficiency.

Acknowledgements: We are sincerely grateful to Jean-Pierre Lecocq for support and valuable discussion, to R.G. Crystal for advice, and to Wilfried Dalemans for transfection technology. The technical assistance of Huguette Schultz and Cecile Chartier is kindly appreciated. We thank Noelle Monfrini for excellent secretarial assistance.

REFERENCES

[1] Carrell, R.W., Jeppson, J.O., Laurell, C.B., Brennan, S.O., Owen, M.C., Vaughan, L. and Boswell, D.R. (1982) Nature 298, 329–334.

[2] Bieth, J.G. (1986) in: Regulation of Matrix Accumulation (Mecham, R ed.) pp. 248–255, Academic Press, New York, NY.

[3] Gadek, J.E. and Crystal, R.G. (1982) in: The Metabolic Basis of Inherited Disease (Stanbury, J.B , Wyngaarden, J.B., Fredrickson, D.S , Goldstein, J.L. and Brown, M.S. eds) pp. 1450–1467, McGraw-Hill, New York, NY

[4] Janoff, A. (1985) Am. Rev. Respir. Dis. 132, 417–433.

[5] Wewers, M D., Casolaro, M.A., Sellers, S E., Swayze, S.C., McPhaul, K.M., Wittes, J.T. and Crystal, R.G. (1987) N. Engl J Med. 316, 1055–1062.

[6] Courtney, M , Buchwalder, A , Tessier, L.H., Jaye, M., Benavente, A , Balland, A., Kohli, V., Lathe, R., Tolstoshev, P. and Lecocq, J P. (1984) Proc. Natl. Acad. Sci. USA 81, 669–673.

[7] Casolaro, M.A., Fells, G., Wewers, M., Pierce, J.E., Ogushi, F., Hubbard, R., Sellers, S., Forstrom, J., Lyons, D., Kawasaki, G. and Crystal, R.G. (1987) J. Appl. Physiol. 63, 2015–2023.

[8] Hubbard, R.C., Casolaro, M.A., Mitchell, M , Sellers, S.E., Arabia, F., Matthay, M.A. and Crystal, R.G. (1988) Proc. Natl. Acad. Sci. USA 86, 680–684.

[9] Hubbard, R.C , McElvaney, N G., Sellers, S.E., Healy, J.T., Czerski, D.B. and Crystal, R.G. (1989) J. Clin Invest. 84, 1349–1354.

[10] Garver, R.I. Jr., Chytil, A., Karlsson, S., Fells, G.A., Brantly, M.L., Courtney, M., Kantoff, P.W., Nienhuis, A.W , Anderson, W.F. and Crystal, R.G. (1987) Proc. Natl. Acad. Sci. USA 84, 1050–1054.

[11] Garver, R.I. Jr , Chytil, A , Courtney, M. and Crystal, R.G. (1987) Science 237, 762–764.

[12] Harrison, T., Graham, F.L. and Williams, J. (1977) Virology 77, 319–329.

[13] Graham, F.L. and Van der Eb, A.J. (1973) Virology 52, 456–467.

[14] Michalski, J.P., McCombs, C.C., Sheth, S., McCarthy, M. and De Shazo, R (1985) J Immunol. Methods 83, 101–112.

[15] Ballay, A., Levrero, M., Tiollais, P and Perricaudet, M. (1987) in: UCLA Symposia on Molecular and Cellular Biology, New Series, Hepadna Viruses, vol. 70 (Robinson, W., Koike, K. and Will, H. eds) pp. 481–493, Alan R. Liss, New York, NY.

[16] Joab, I., Rowe, D.T., Bodescot, M., Farrell, P.J. and Perricaudet, M. (1987) J Virol. 61, 3340–3344.

[17] Levrero, M., Jean-Jean, O., Balsano, F., Will, H. and Perricaudet, M. (1990) Virology 174, 299–304.

[18] Tosoni-Pittoni, E., Joab, I., Nicolas, J C. and Perricaudet, M. (1989) Biochem. Biophys Res. Commun 158, 676–684.

[19] Jean-Jean, O., Levrero, M., Will, H., Perricaudet, M. and Rossignol, J.M. (1989) Virology 170, 99–106.