REFERENCES


Transfer and Expression of Neutrophil Inhibitory Factor Gene in Endothelial and Epithelial Cells Prevent Neutrophil Adhesion*

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Neutrophil (polymorphonuclear leukocyte [PMN])-dominated inflammation in the lung contributes to the pathophysiology of such disorders as cystic fibrosis.

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chronic bronchitis, idiopathic pulmonary fibrosis, and the adult respiratory distress syndrome. PMN adhere, via β2 integrins, to intercellular adhesion molecule-1 (ICAM-1) of both endothelial and epithelial cells. ICAM-1 is upregulated on these cells in response to inflammatory stimuli. We hypothesized that transfer and expression to these cells of the canine hookworm neutrophil inhibitory factor (NIF) protein, which binds the I domain of CD11b of the leukocyte CD11b/CD18 β2 integrin and blocks its interaction with ICAM-1, might prevent PMN adhesion.

To evaluate this hypothesis, the NIF cDNA was subcloned into the plasmid pLNCX, 3′ of the cytomegalovirus (CMV) immediate early promoter, and this retroviral vector was stably integrated into endothelial cells (EAhy.929 or HMEC). After G418 selection, NIF mRNA transcripts were documented in the transduced cells by Northern blot analysis, and NIF protein in supernatants by immunoprecipitation and an enzyme-linked immunosorbent assay (ELISA). The ability of calcein acetoxymethyl ester-loaded PMN to adhere to endothelial cells was evaluated with a fluorescent plate reader.

NIF-expressing cells exhibited 80 to 90% decreased adhesion to phorbol ester-stimulated PMN (compared to nontransduced control cells, p<0.01) and conditioned media from transduced cells reduced the adhesion of nontransduced cells. NIF expressing stimulated with tumor necrosis factor-alpha (TNF-α) (1,000 U/mL) exhibited approximately 80% decreased adhesion to PMN (as compared to TNF-α-stimulated, nontransduced control cells, p<0.01). The decrease in PMN adhesion was abrogated in both situations by an anti-NIF polyclonal antibody.

To more closely approach a gene transfer strategy that would be applicable in vivo, a replication-deficient recombinant adenovirus (E1−, E3−) was constructed containing the NIF cDNA under the control of the CMV promoter (AdCMV-NIF). After in vitro adenovirus-mediated transfer and expression of the NIF cDNA into mouse lung epithelial cells (MLE), NIF protein was detected by ELISA from supernatants above the cells. A 60 to 90% reduction of PMN adhesion to TNF-α-stimulated (1,000 U/mL), AdCMV-NIF-infected MLE (p<0.01 compared to control cells) was documented. That this was an NIF effect was again confirmed by abrogation of the reduced adhesion with the anti-NIF antibody.

These data indicate that PMN adherence to lung epithelial and endothelial cells is mediated in part by CD11b and suggest a strategy of gene transfer and expression that may be of value in amelioration of lung disease involving PMN-dependent pathophysiology.

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Glycosylated Polylysines*

Nonviral Vectors for Gene Transfer Into Cystic Fibrosis Airway Epithelial Cells

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Vir al vectors, although efficient for the transfection of the cystic fibrosis gene (CFTR), have been found to be immunogenic in cytosic fibrosis (CF) patients, and therefore other vectors are being sought.1 Carbohydrates conjugated to polysine offer an alternative to viral vectors and have several of the following advantages: (1) targeting specific cell types by binding to endogenous lectins on the cell surface; (2) condensing DNA in small particles to facilitate the passage through the cell membrane; and (3) producing no immune response. Based on the use of glycosylated polysine, Erbacher et al2 described a gene delivery system for HepG2 cells, which express a galactose-specific lectin. Little information is available on the endogenous lectins of human airway epithelial cells. Therefore, studies were initiated using different glycosylated polylysines to deliver genes to immortalized and primary CF airway epithelial cells.

To determine gene transfer and expression, the reporter gene luciferase was used with a cytomegalovirus promoter. The plasmid was complexed to different glycosylated polylysines and added to an immortalized CF cell line (CF/T43, ΔF508/ΔF508) or to primary CF and non-CF cells in culture for 4 h at 37°C in the presence of chloroquine. After 48 h in growth medium, the cells were lysed. Luciferase gene expression was measured in a luminometer and reported as relative light units (RLU) per milligram of protein.

Lactosylated polysine was highly effective in the transfer of the luciferase gene into CF/T43 cells (4×106 RLU per milligram of protein). In primary CF cells, lactosylated polysine was the most effective vector (4×106 RLU per milligram of protein). Thus, CF airway epithelial cells may contain an endogenous lectin of the galectin type.3 It is anticipated that lactosylated polysine will serve as a useful vector to transfer CFTR into CF airway epithelial cells.

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