FEBS Letters 357 (1995) 140–144 FEBS 14963

Adhesion molecules: a new target for immunoliposome-mediated drug delivery

P.G.M. Bloemen[a,*], P.A.J. Henricks[a], L. van Bloois[b], M.C. van den Tweel[a], A.C. Bloem[c], F.P. Nijkamp[a], D.J.A. Crommelin[b], G. Storm[b]

[a]*Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80.082, 3508 TB Utrecht, The Netherlands*
[b]*Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80.082, 3508 TB Utrecht, The Netherlands*
[c]*Department of Immunology, Academic Hospital Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands*

Received 25 November 1994

Abstract The anti-ICAM-1 monoclonal antibody F10.2 was conjugated to liposomes to target to cells expressing the cell adhesion molecule ICAM-1. We demonstrate that F10.2 immunoliposomes bind to human bronchial epithelial cells (BEAS-2B) and human umbilical vein endothelial cells (HUVEC) in a specific, dose- and time-dependent manner. It appears that the degree of ICAM-1 expression is the limiting factor in the degree of immunoliposome binding to the cells. These results are a first step in the strategy for specific drug delivery to target sites characterised by increased expression of adhesion molecules.

Key words: Immunoliposome; Monoclonal antibody; ICAM-1; Epithelium; Endothelium

1. Introduction

Cell adhesion molecules are (glyco)proteins which are expressed on the cell membrane and are involved in homotypic and heterotypic cell interactions. Since cell–cell interactions are crucial in pathophysiological events, adhesion molecules play an important role in processes like wound healing, tumor metastasis formation, lymphocyte homing, and lymphocyte and granulocyte extravasation [1–5]. Evidence is accumulating that the expression of particular cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin and/or vascular cell adhesion molecule-1 (VCAM-1), is locally induced or enhanced at areas of inflammation [6]. These adhesion molecules mediate the invasion of leukocytes into extravascular tissue at pathological sites of e.g. rheumatoid arthritis [7,8], atopic dermatitis [9], inflammatory bowel diseases [10], and asthma [11,12]. Induction and/or increased expression of certain cell adhesion molecules at inflammatory loci associated with various diseases offers opportunities for the development of new therapeutic strategies. Monoclonal antibodies (mAbs) against adhesion molecules have been used to inhibit leukocyte recruitment into areas of dermal, synovial and lung inflammation [13–16], to increase survival in animal models of septic shock [17], and to prevent ameliorate tissue damage caused by ischemia-reperfusion injury [18]. Another opportunity for therapeutic intervention is to couple these mAbs to liposomes (immunoliposomes) for the purpose of drug targeting. This study deals with the use of an anti-ICAM-1 mAb to direct liposomes to target cells expressing the adhesion molecule ICAM-1. This is a first step in the strategy for specific delivery of bioactive substances to target sites characterized by increased expression of adhesion molecules.

Liposomes conjugated with mAbs (referred to as immunoliposomes) offer several advantages over direct mAb–drug conjugates [19]. First, large quantities of a wide range of polar and lipophilic drugs can be encapsulated in liposomes, while usually not more than a few drug molecules can be directly coupled to soluble drug–antibody conjugates. Therefore, the relatively large pay-load per immunoliposome permits the transfer of large numbers of drug molecules to an individual target site. Secondly, encapsulation does not require linking of the drug to the targeting ligand via chemical bonds, which must be degraded before drug activity can be displayed. Moreover, the immunoliposome particle protects the drug from metabolic degradation until it reaches its destination. Finally, many mAbs of a single or multiple different specificities can be bound to the surface of a single liposome. Such immunoliposomes can bind to cells expressing the target antigen by multivalent interactions [20,21].

Immunoliposomes specifically directed against the adhesion molecule ICAM-1 were prepared, using the anti-ICAM-1 mAb F10.2 [22], characterised and evaluated for their in vitro binding capacity to cells expressing ICAM-1. As target cells we used human umbilical vein endothelial cells (HUVEC) and the bronchial epithelial cell line BEAS-2B, which are well characterized for their ICAM-1 expression [23,24]. We show that the F10.2-mediated targeting of liposomes to ICAM-1 expressing cells is rapid, specific and positively correlated with the degree of ICAM-1 expression. The use of antibodies to target liposomes to cell adhesion molecules has not been reported previously.

2. Materials and methods

2.1. Materials

2.1.1. Materials for liposome preparation. Partially hydrogenated egg-phosphatidyl choline with an iodine value of 40 (PHEPC; Asahi Chemical Industry Co., Ibarakiken, Japan) was prepared as described previously [25]. Egg-phosphatidylglycerol (EPG) was a gift from

*Corresponding author. Fax: (31) (30) 537 420.

Abbreviations: BSA, bovine serum albumine; CHOL, cholesterol; DMF, dimethylformamide; EDTA, ethylene diaminetetraacetic acid; EPG, egg-phosphatidylglycerol; FCS, foetal calf serum; GamFITC, fluorescein isothiocyanate-labeled goat anti-mouse mAb; HBS, HEPES-buffered saline; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; MPB-PE, *N*-[4-(*p*-maleimidophenyl)butyryl] phosphatidylethanolamine; MFI, mean fluorescence intensity; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PHEPC, partially hydrogenated egg L-α-phosphatidylcholine; PL, phospholipid; SATA, *N*-succinimidyl *S*-acetylthioacetate; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

SSDI 0014-5793(94)01350-0

Nattermann GmbH (Cologne, Germany). Rhodamine-PE was obtained from Lipid Products (South Nutfield, UK), *N*-succinimidyl-*S*-acetylthioacetate (SATA) from Pierce Chemical Co. (Rockford, IL, USA), cholesterol (CHOL) and *N*-ethylmaleimide from Sigma Chemical Co. (St Louis, MO, USA), and dimethylformamide (DMF) and hydroxylamine from Janssen (Beerse, Belgium).

2.1.2. Sources for mediators and mAbs. Tumor necrosis factor-α (TNF-α; Genzyme Corp., Cambridge, MA, USA); interferon-γ (IFN-γ; Boehringer-Mannheim GmbH, Mannheim, Germany); keratinocyte medium (Keratinocyte-SFM; Gibco, Grand Island, NY, USA); RPMI 1640 (Gibco); vitrogen (Celtrix Laboratories, Palo Alto, CA, USA), fibronectin (Centraal Laboratorium Bloedtransfusiedienst (CLB), Amsterdam, The Netherlands); heat-inactivated pooled human serum (CLB); bovine serum albumin (BSA; fraction V) with less than 0.1 ng/mg endotoxin (Sigma); FITC-labeled goat anti-mouse mAb (GamFITC; Becton-Dickinson Monoclonal Center, Mountain View, CA, USA) and anti-ICAM-1 mAb (IgG_1), clone F10.2 [22]. The mAbs anti-ICAM-1 (IgG_{2a}), clone R6.5 and anti-CD18 (IgG_1), clone R15.7 were kindly donated by Dr. C.D. Wegner (Department of Pharmacology, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA). All other reagents were of the highest grade available.

2.2. Cell cultures

The human bronchial epithelial cell line BEAS-2B [26] was obtained from Dr. J.F. Lechner (National Institute of Health, Bethesda, MD, USA) and cultured as described by Bloemen et al. [23]. Briefly, BEAS-2B cells were cultured in a serum-free keratinocyte medium with 50 μg/ml gentamycin on a coat of vitrogen, fibronectin and BSA. The cell line was not used after passage 30.

Human umbilical cord vein endothelial cells (HUVEC; kind gift of Dr. J. de Vlieger, Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands) were isolated from human umbilical cord vein and cultured in fibronectin coated plastic culture flasks (Costar, Cambridge, MA, USA) using RPMI 1640 supplemented with 20% heat-inactivated pooled human serum, penicillin (100 U/ml) and streptomycin (100 μg/ml).

The murine CT4S cell line (kindly donated by Dr. W.P. Paul, National Institute of Health, Bethesda, MD, USA) was cultured in RPMI 1640 with 10% foetal calf serum (FCS).

The cell lines were free of mycoplasma contamination and cultured at 37°C with 5% CO_2 in humidified air.

2.3. Preparation of thiolated mAb

The F10.2 mAb was eluted from a gel-filtration column (Sephadex G-25M, PD-10) with HEPES buffer (10 mM HEPES, 135 mM NaCl, 1 mM EDTA) and subsequently concentrated (10 kDa Microsep; Filtron Technology Corp., Northborough, MA, USA) to a standard concentration of about 1 mg protein/ml. Thiol groups were introduced at the site of primary amino groups in the molecule by using the SATA reaction proposed by Duncan and co-workers [27]. SATA was dissolved in DMF at a concentration of 5 mM. The solutions were mixed in a volume ratio of SATA/F10.2 mAb = 1/100. They were incubated at room temperature for 20 min at pH 7.4 under constant rotation with a SATA/F10.2 mAb molar ratio of 8:1. Immediately after incubation, acetylthioacetyl-protein (protein-ATA) was separated from unreacted reagent by gel-filtration on a Sephadex G-25M column (PD-10). The protein fractions in the eluate were detected by monitoring the absorption at 280 nm. The F10.2 mAb containing fractions were combined and stored at −20°C. Before coupling to MPB-PE liposomes (see below), protein-ATA was deacetylated by adding a freshly prepared 0.5 M hydroxylamine-HCl solution containing 0.5 M HEPES, 25 mM EDTA (pH 7.5) at a volume ratio of protein-ATA solution/NH_2OH solution = 10/1; the incubation lasted 1 h.

2.4. Preparation of immunoliposomes

Immunoliposomes were prepared by conjugating the anti-ICAM-1 mAb F10.2 to preformed liposomes containing the anchor molecule MPB-PE. *N*-[4-(*p*-Maleimidophenyl) butyryl]phosphatidylethanolamine (MPB-PE) was synthesized, purified and analyzed as described before [28,29]. MPB-PE was incorporated into the liposomal bilayers to allow covalent coupling of thiol containing proteins to the liposomal surface [30–33]. The lipid composition of the liposomes used was PHEPC/EPG/CHOL/MPB-PE/rhodamine-PE at a molar ratio of 10/1/4/0.4/0.01. A mixture of the appropriate amounts of lipids in chloroform/methanol (10/1, v/v) was evaporated to dryness by rotary evaporation at 35°C under reduced pressure. After flushing with nitrogen, the lipid film was hydrated in HEPES buffer. The resulting liposome dispersion was sequentially extruded through polycarbonate membrane filters of 0.6 and 0.2 μm pore size (Unipore, Bio-Rad, Richmond CA, USA) under nitrogen pressure up to 0.8 MPa. The freshly prepared liposomes were mixed with freshly deacylated, thiolated F10.2 mAb (concentrations during incubation amounted to about 10 μmol phospholipid (PL)/ml and 0.25 mg mAb/ml, respectively). The coupling reaction was allowed to take place for 75 min at room temperature under constant rotation in nitrogen atmosphere. The coupling reaction was stopped by adding 50 μl of *N*-ethylmaleimide (8 mM in HEPES buffer). Finally, the immunoliposomes were separated from unconjugated antibody by ultracentrifugal sedimentation at 100,000 × *g* for 30 min at 4°C. The pellet was resuspended and washed three times with HEPES buffer. MPB-PE-containing liposomes not incubated with thiolated mAb are referred to as 'MPB-PE liposomes'. Liposomes without incorporated MPB-PE are referred to as 'liposomes'. Liposome dispersions were stored at 4°C.

2.5. Liposome characterisation

Lipid phosphate was determined by the colorimetric method of Fiske and Subbarow [34]. The amount of protein coupled to the liposomes was determined by the method of Wessel and Flügge [35], with BSA as standard. The amount of mAb coupled to the liposomes was expressed as μg protein/μmol PL. The protein coupling ratios of the F10.2 immunoliposomes varied between 4 and 8 μg protein/μmol PL. Mean particle size was determined by dynamic light scattering with a Malvern 4700 system using a 25 mW helium–neon laser and the AUTOMEASURE vsn. 3.2 software (Malvern Ltd., Malvern, UK). For viscosity and refractive index the values of pure water were used. As a measure of the particle size distribution of the dispersion the system reports a polydispersity index. This index ranges from 0.0 for an entirely monodisperse up to 1.0 for a completely polydisperse dispersion. The liposomes had a mean size of 0.25–0.30 μm and a polydispersity index varying between 0.1 and 0.3. It can be estimated that 40–80 mAb molecules were coupled to one liposome particle.

2.6. Analysis of ICAM-1 expression

Confluent monolayers of BEAS-2B cells and HUVEC (non-stimulated or stimulated for 24 h by pretreatment with IFN-γ (200 U/ml) and TNF-α (20 ng/ml), respectively) were detached using HEPES-buffered saline (HBS), EDTA (0.02%) for 10–15 min. CT4S cells were detached by use of a cell scraper. The different cell types were subsequently washed with phosphate buffered saline (PBS), 0.1% BSA, 0.05% sodium azide. Aliquots of 5×10^5 cells were incubated with the anti-ICAM-1 mAb F10.2 at a concentration of 25 μg/ml (incubation volume 50 μl) for 30 min on ice. Subsequently, they were incubated for another 30 min with a FITC-conjugated goat anti-mouse IgG (GamFITC). Finally, the cells were washed 3 times and analyzed by flowcytometry under identical settings for all cell types (FACScan, Becton-Dickinson). The ICAM-1 expression is expressed as fluorescence intensity (*x*-axis) plotted on a log scale vs. cell number (*y*-axis). The mean fluorescence intensity (MFI) was calculated from these histograms and used in the figures. The MFI of the negative control (GamFITC labeling only) was ≤ 3 for BEAS-2B cells and ≤ 12 for HUVEC.

2.7. Liposome binding assay

Detached BEAS-2B cells (see section 2.6.) were washed in Keratinocyte-SFM medium and detached HUVEC and CT4S cells in RPMI 1640. Resuspended cells (5×10^5) were incubated with varying lipid concentrations of liposomes, MBP-PE liposomes or F10.2 immunoliposomes in a total volume of 200 μl for 0–4 h at 37°C. The cell/liposome suspensions were washed twice with PBS, 0.1% BSA, 0.05% sodium azide to remove unbound liposomes before analysis on the flowcytometer on identical settings as mentioned before. Intensity of liposome binding as monitored by the presence of rhodamine-PE (λ_{exc} = 560 nm, λ_{em} = 590 nm) in the liposome was expressed as fluorescence intensity (*x*-axis) plotted on a log scale histogram vs. cell number (*y*-axis). The MFI were calculated from these histograms and used in the figures. In the liposome binding inhibition experiments, 5×10^5 epithelial cells or HUVEC were preincubated with different mAbs (F10.2, R6.5, or R15.7) at a concentration of 25 μg/ml (incubation volume 50 μl) for 30 min on ice. Subsequently, the cells were washed and incubated with the liposome suspension for 1 h at 37°C.

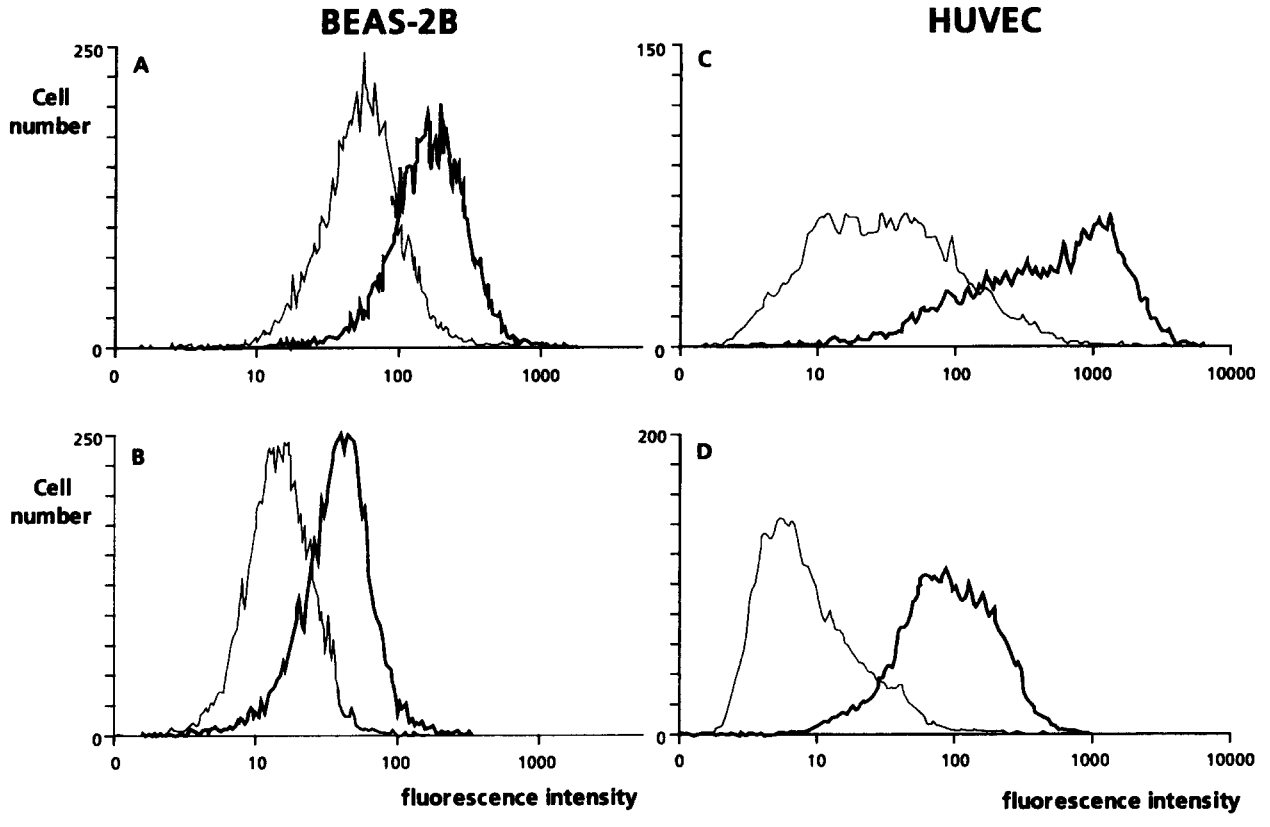

Fig. 1. ICAM-1 expression (A,C) and F10.2 immunoliposome binding (B,D) to BEAS-2B cells and HUVEC. The cells were detached after 24 h of incubation without cytokines (thin lines) or with IFN-γ for BEAS-2B cells and TNF-α for HUVEC (dark lines). 5×10^5 cells (A,C) were incubated with the anti-ICAM-1 mAb F10.2 (at a concentration of 25 μg/ml) and subsequently wih GamFITC. In parallel, BEAS-2B cells and HUVEC were incubated with F10.2 immunoliposomes at a concentration of 6 μmol PL/ml for 1 h at 37°C (B,D). Representative examples of ICAM-1 expression (A,C) and liposome binding (B,D) as measured by flowcytometry are shown. Binding of the control liposomes; i.e. 'liposomes' and MBP-PE liposomes gave a MFI $\leq$ 7 on BEAS-2B cells and a MFI $\leq$ 19 on HUVEC, independent of the activation state of the cell.

3. Results and discussion

As specific association of immunoliposomes with their target cells is a necessary requirement for target-specific drug delivery, the binding of F10.2 immunoliposomes, containing rhodamine-PE as fluorescent bilayer marker, to BEAS-2B cells and HUVEC was evaluated. Both cell types expressed ICAM-1 on their surface under normal culture conditions (Fig. 1A,C; MFI = 152 for BEAS-2B cell and MFI = 57 for HUVEC). ICAM-1 expression on BEAS-2B cells was increased 2-fold (MFI = 302) and on HUVEC 13-fold (MFI = 723) after incubation for 24 h with IFN-γ and TNF-α, respectively (Fig. 1A,C). Binding of the F10.2 immunoliposomes to the cells was monitored by flowcytometry after 1 h of incubation at 37°C. The F10.2 immunoliposomes appeared to bind to both BEAS-2B cells and HUVEC under non-stimulated conditions (MFI = 30 and 13, respectively). The amount of liposomes bound was clearly increased on BEAS-2B cells (MFI = 62) and on HUVEC (MFI = 117) after up-regulation of the surface expression of ICAM-1 by the respective cytokine pretreatment (Fig. 1B,D).

Fig. 2 shows the effect of the incubation time on the degree

Table 1
Inhibition of F10.2 immunoliposome cell binding by preincubation of BEAS-2B and HUVEC with soluble anti-ICAM-1 mAbs

	No pre-incubation (MFI)			Pre-incubation with F10.2 (MFI)			Pre-incubation with R6.5 (MFI)		Pre-incubation with R15.7 (MFI)	
Experiment	I	II	III	I	II	III	I	II	I	II
BEAS-2B (NS)	16	14	12	9	7	4	9	7	16	18
BEAS-2B (IFN-γ)	39	31	37	17	8	16	16	12	42	25
HUVEC (NS)	13	16	ND	14	9	ND	ND	ND	ND	ND
HUVEC (TNF-α)	117	48	ND	33	14	ND	ND	ND	ND	ND

BEAS-2B cells and HUVEC were respectively incubated with or without IFN-γ or TNF-α for 24 h. After detachment, aliquots of 5×10^5 cells were incubated with control medium, R15.7 (anti-CD18), R6.5 or F10.2 (anti-ICAM-1) for 30 min on ice. After washing, these cells were subsequently incubated with F10.2 immunoliposomes at a concentration of 6 μmol PL/ml for 1 h at 37°C. Liposome binding was analyzed with a flowcytometer and expressed as MFI. Experiment I, II and III represent the data of experiments performed with seperate immunoliposome formulations. NS, not stimulated; ND, not done.

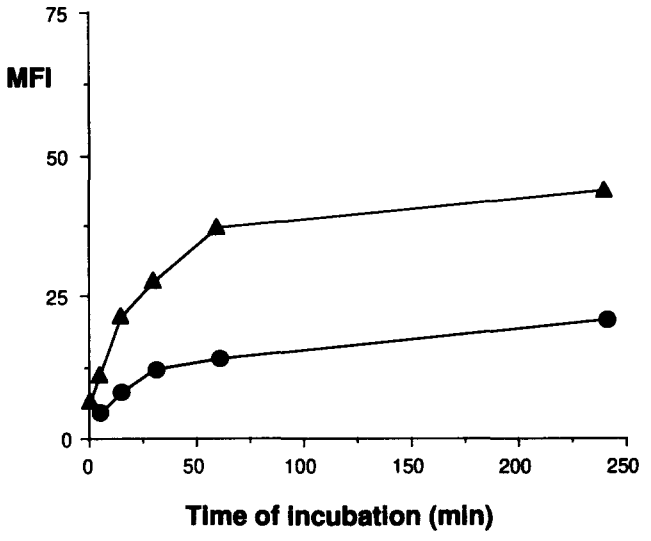

Fig. 2. Time-course of liposome binding to BEAS-2B cells. 5×10^5 non-stimulated (circles) or IFN-γ stimulated (triangles) cells were incubated at 37°C with F10.2 immunoliposomes at a concentration of 1.2 μmol PL/ml. These experiments were carried out with 2 different liposome preparations. Results of a typical experiment are shown.

of liposome binding to BEAS-2B cells. Under the chosen conditions, cell binding to non-stimulated and IFN-γ stimulated cells was nearly maximal after 1 h of incubation, demonstrating that an incubation period of 1 h was sufficient. The time-curve was similar for the different liposome preparations studied and therefore independent of the coupling ratio.

Fig. 3 shows that the number of cell-bound immunoliposomes increased with the liposomal lipid concentration in the incubation mixture. Clearly, the degree of liposome binding to the cells was increased after incubation of the BEAS-2B cells with IFN-γ at all lipid concentrations tested. Similar concentration curves were found with the different liposome preparations.

To show the specificity of the F10.2 immunoliposome binding towards ICAM-1 expressing cells, negative control binding experiments were performed using MPB-PE liposomes and liposomes without incorporated MPB-PE ('liposomes'). These negative control incubations resulted in very low binding values even at high lipid concentrations (Fig. 3). In addition, F10.2 immunoliposomes did not bind to murine CT4S cells which do not express ICAM-1 recognised by F10.2, as confirmed by flowcytometry (MFI = 2 for ICAM-1 expression and MFI = 4 for liposome binding). In line with these results, F10.2 immunoliposome binding to neutrophils, monocytes or lymphocytes was not detectable, which corresponds with the low ICAM-1 expression on leukocytes in comparison with the endothelial and epithelial cells used (data not shown). To further demonstrate the specific nature of the immunoliposome–cell interaction, experiments were designed to study whether this interaction could be blocked by preincubation of the target cells with soluble mAb directed against ICAM-1. Although directed against different epitopes on ICAM-1 [22,36,37], the mAbs F10.2 and R6.5 both inhibited the binding of F10.2 immunoliposomes to non-stimulated and stimulated BEAS-2B cells (Table 1). Steric hindrance might be an explanation for the inhibition of F10.2 immunoliposome binding by the mAb R6.5. Preincubation of the cells with an irrelevant isotype matched mAb R15.7 (anti-CD18) had no inhibitory effect (Table 1). Identical experiments with HUVEC yielded similar results (Table 1).

The relationship between the degree of ICAM-1 expression on the different cell types and the amount of liposomes bound to these cells is shown in Fig. 4. Linear regression analysis revealed a highly significant positive correlation ($r = 0.975$, $P < 0.001$ for experiment I, and $r = 0.956$, $P < 0.05$ for experiment II). It appears that the degree of ICAM-1 expression is the limiting factor in the degree of liposome binding to the cells. In addition, it is also clear from Fig. 4 that the degree of F10.2 immunoliposome binding is dependent on the number of mAb molecules present on the surface of the liposomes. Together, these results (Figs. 3, 4, and Table 1) indicate that F10.2 immunoliposomes bind specifically to cells expressing ICAM-1.

It should be realised that the present experimental design to monitor cell binding of liposomes does not discriminate between liposome binding to the cell surface and uptake into the cell. The subsequent events which follow liposome cell binding via the ICAM-1/mAb interaction are currently under investigation. Since the fate of the liposome largely dictates the routing, and therefore action, of an encapsulated drug, the nature of these events have to be elucidated.

In conclusion, we have demonstrated for the first time the

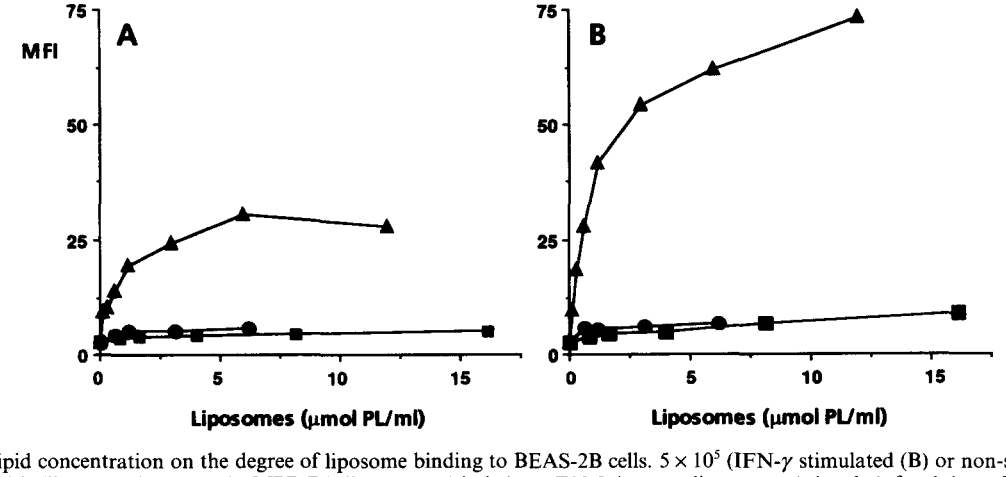

Fig. 3. Effect of lipid concentration on the degree of liposome binding to BEAS-2B cells. 5×10^5 (IFN-γ stimulated (B) or non-stimulated (A)) cells were incubated with 'liposomes' (squares), MPB-PE liposomes (circles) or F10.2 immunoliposomes (triangles) for 1 h at 37°C ($n = 3$). These experiments were carried out with 3 different liposome preparations. Results of a typical experiment are shown.

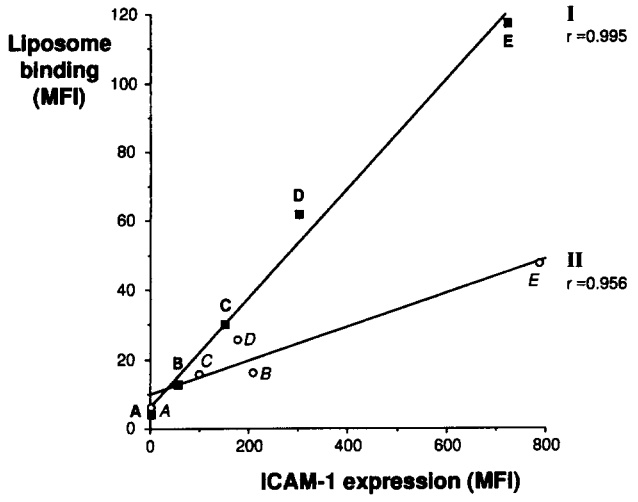

Fig. 4. Effect of degree of ICAM-1 expression on the degree of cell binding of F10.2 immunoliposomes. The degree of ICAM-1 expression and the degree of cell binding of F10.2 immunoliposomes were determined for different cell populations and two immunoliposome formulations differing in coupling ratio (7.7 μg protein/μmol PL for experiment I and 4.2 μg protein/μmol PL for experiment II). The following cells were used: A, CT4S; B, HUVEC (non-stimulated); C, BEAS-2B (non-stimulated); D, BEAS-2B (IFN-γ) and E, HUVEC (TNF-α). 5×10^5 cells (BEAS-2B, HUVEC, and CT4S) were incubated with F10.2 immunoliposomes at a PL concentration of 6 μmol PL/ml for 1 h at 37°C. ICAM-1 expression expressed as MFI (x-axis) is plotted against F10.2 immunoliposome binding to the different cells expressed as MFI (y-axis) ($r = 0.975$, $P \leq 0.001$ for experiment I, and $r = 0.956$, $P < 0.05$ for experiment II).

potential usefulness of immunoliposomes for targeted drug delivery to cells expressing adhesion molecules. Immunoliposomes specifically directed against cells expressing ICAM-1 were prepared, characterized and shown to be capable of binding specifically to target cells from different origins in vitro. Moreover, the results presented in this study open up a new avenue in drug delivery research; i.e immunoliposomes containing drugs can be targeted to a pathological site characterized by an increased expression of certain adhesion molecules and act as a localized drug depot. Further studies will address the many remaining issues such as extent of cellular internalization of cell-bound immunoliposomes, the quantification of the degree of liposome binding to the cell, the general applicability of this approach for targeting to other types of cell adhesion molecules (such as E-selectin, P-selectin or VCAM-1 [1,9,38]) only expressed at the site of an inflammatory process and totally absent on blood leukocytes, and the validation of the concept in suitable in vivo models.

Acknowledgments This work was supported in part by the Dutch Asthma Foundation

References

[1] Springer, T.A. (1990) Nature 346, 425–434.
[2] Kuypers, T.W. and Roos, D. (1989) Res. Immunol. 140, 461–486.
[3] Kishimoto, T.K., Larson, R.S., Corbi, A.L., Dustin, M.L., Staunton, D.E. and Springer, T. (1989) Adv. Immunol. 46, 149–181.
[4] Gorski, A. (1994) Immunol. Today 15, 251–255.
[6] Pober, J.S. and Cotran, R.S. (1990) Transplantation 50, 537–544.
[7] Koch, A.E., Burrows, J.C., Haines, G.K., Carlos, T.M., Harlan, J.M. and Leibovich, S.J. (1991) Lab. Invest. 64, 313–320.
[8] Hale, L.P., Martin, M.E., McCollum, D.E., Nunley, J.A., Springer, T.A., Singer, K.H. and Haynes, B.F. (1989) Arthritis Rheum. 32, 22–30.
[9] Kyan-aung, U., Haskard, D.O., Poston, R.N., Thornhill, M.H. and Lee, T.H. (1991) J. Immunol. 146, 521–528.
[10] Malizia, G., Calabrese, A., Cottone, M., Raimondo, M., Trejdosiewicz, L.K., Smart, C.J., Oliva, L. and Pagliaro, L. (1991) Gastroenterology 100, 150–159.
[11] Wegner, C.D., Gundel, R.H., Reilly, P., Haynes, N., Letts, L.G. and Rothlein, R. (1990) Science 247, 456–459.
[12] Leff, A.R., Hamann, K.J. and Wegner, C.D. (1991) Am. J. Physiol. 260, L189–L206.
[13] Forrest, M.J., Eiermann, G.J., Meurer, R., Walakovits, L.A. and MacIntyre, D.E. (1992) Br. J. Pharmacol. 106, 287–294.
[14] Gundel, R.H., Wegner, C.D., Torcellini, C.A., Clarke, C.C., Haynes, N., Rothlein, R., Smith, C.W. and Letts, L.G. (1991) J. Clin. Invest. 88, 1407–1411.
[15] Weg, V.B., Williams, T.J., Lobb, R.R. and Nourshargh, S. (1993) J. Exp. Med. 177, 561–566.
[16] Gundel, R.H., Wegner, C.D., Torcellini, C.A. and Letts, L.G. (1992) Clin. Exp. Allergy 222, 569–575.
[17] Jaeschke, H., Farhood, A. and Smith, C.W. (1991) Am. J. Physiol. 261, G1051–G1056.
[18] Rosen, H. and Gordon, S. (1989) Br. J. Exp. Pathol. 70, 385–394.
[19] Vingerhoeds, M.H., Storm, G. and Crommelin, D.J.A. (1994) Immunomethods 4 (in press).
[20] Bragman, K.S., Heath, T.D. and Papahadjopoulos, D. (1983) Biochim. Biophys. Acta 730, 187–195.
[21] Heath, T.D., Fraley, R.T., Bentz, J., Voss, E.W., Herron, J.N. and Papahadjopoulos, D. (1984) Biochim. Biophys. Acta 770, 148–158.
[22] Bloemen, P., Moldenhauer, G., Van Dijk, M., Schuurman, H. and Bloem, A.C. (1992) Scand. J. Immunol. 35, 517–523.
[23] Bloemen, P.G.M., Van den Tweel, M.C., Henricks, P.A.J., Engels, F., Wagenaar, S.S., Rutten, A.A.J.J.L. and Nijkamp, F.P. (1993) Am. J. Respir. Cell Mol. Biol. 9, 586–593.
[24] Pober, J.S., Gimbrone, M.A., Lapierre, L.A., Mendrick, D.L., Fiers, W., Rothlein, R. and Springer, T.A. (1986) J. Immunol. 137, 1893–1896.
[25] Lang, J., Vigo-Pelfrey, C. and Martin, F. (1990) Chem. Phys. Lipids 53, 91–101.
[26] Reddel, R.R., Ke, Y., Gerwin, B.I., McMenamin, M.G., Lechner, J.F., Su, R.T., Brash, D.E., Park, J., Rhim, J.S. and Harris, C.C. (1988) Cancer Res. 48, 1904–1909.
[27] Duncan, R.J.S., Weston, P.D. and Wrigglesworth, R. (1983) Anal. Biochem. 132, 68–73.
[28] Martin, F.J. and Papahadjopoulos, D. (1982) J. Biol. Chem. 257, 286–288.
[29] Nassander, U.K., Steerenberg, P.A., De Jong, W.H., Overveld, van W.O.W.M., Te Boekhorst, C.M.E., Poels, L.G., Jap, P.H.K. and Storm, G. (1994) Biochim. Biophys. Acta (in press).
[30] Vingerhoeds, M.H., Haisma, H.J., van Muijen, M., van de Rijt, R.B.J., Crommelin, D.J.A. and Storm, G. (1993) FEBS Lett. 336, 485–490.
[31] Storm, G., Nassander, U.K., Steerenberg, P.A., Vingerhoeds, M.H. and Crommelin, D.J.A. (1994) J. Liposome Res. 4, 641–666.
[32] Blume, G., Cevc, G., Crommelin, D.J.A., Bakker-Woudenberg, I.A.J.M., Kluft, C. and Storm, G. (1993) Biochim. Biophys. Acta 1149, 180–184.
[33] Heeremans, J.L.M., Kraaijenga, J.J., Los, P., Kluft, C. and Crommelin, D.J.A. (1992) Biochim. Biophys. Acta 1117, 258–264.
[34] Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400.
[35] Wessel, D. and Flügge, U.I. (1984) Anal. Biochem. 138, 141–143.
[36] Staunton, D.E., Dustin, M.L., Erickson, H.P. and Springer, T.A. (1990) Cell 61, 243–254.
[37] Diamond, M.S., Staunton, D.E., Fougerolles, de A.R., Stacker, S.A., Gracia-Aguilar, J., Hibbs, M.L. and Springer, T.A. (1990) J. Cell Biol. 111, 3129–3139.
[38] Pelletier, R.P., Ohye, R.G., Vanbuskirk, A., Sedmak, A.D., Kincade, P., Ferguson, R.M. and Orosz, C.G. (1992) J. Immunol. 149, 2473–2481.