Transport of Biologically Active Interferon-gamma Across Human Skin *in Vitro*

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Purpose. Several studies have suggested epidermal uptake of cytokines, such as interferons, can be facilitated using topical liposomal formulations. We have evaluated the *in vitro* transport of biologically active recombinant human interferon-γ (rhIFN-γ) into and through split-thickness human skin to assess this possibility. ***Methods.*** Skin samples were exposed to rhIFN-γ under various conditions involving hydrated and dry surface conditions in the presence and absence of liposomes. A new low-level ELISA and an anti-viral bioassay were used to quantitate transported rhIFN-γ. Immunohistochemical staining for ICAM-1 expression by keratinocytes was used to visualize the extent and distribution of rhIFN-γ transport. ***Results.*** Apparent steady-state transport of rhIFN-γ occurred within the first 5 hours of exposure with approximately 10% of transported rhIFN-γ demonstrating bioactivity. While the permeability of rhIFN-γ across human skin under drying conditions was enhanced by the presence of liposomes, no augmentation of permeability was observed when the skin was kept hydrated. Liposomal formulations of rhIFN-γ had greater transport rates than aqueous formulations when the applied formulations were allowed to dry after dosing. ***Conclusions.*** Our results demonstrate the transport of biologically active rhIFN-γ across human skin in vitro and suggest a role for stratum corneum hydration as one possibility for the augmented cytokine transport.

KEY WORDS: cytokine; liposomes; topical delivery; intercellular adhesion molecule-1 (ICAM-1); recombinant human interferon-gamma (rhIFN-γ); enzyme-linked immunoadsorbent assay (ELISA); bioassay; *in vitro*.

INTRODUCTION

Clinical studies have suggested recombinant human interferon-γ (rhIFN-γ) to be of benefit in at least two skin conditions: atopic dermatitis [1] and keloidal scarring [2]. Atopic dermatitis, a chronic inflammatory skin disease, is associated with elevated immunoglobulin E serum levels and depressed IFNγ levels [3]. Keloid formation results from an over-production of collagen at wound sites, and the ability of rhIFN-γ to suppress fibroblast collagen synthesis [2] might be the basis for its effectiveness. Systemic administration of this 16,440 molecular weight cytokine, however, can produce significant side effects [4, 5]. One approach to possibly lessen or even obviate these side effects in the treatment of skin conditions would be to deliver rhIFN-γ topically.

The hydrophobic nature of the stratum corneum provides the primary barrier to the transport of topically applied water-soluble drugs [6–8]. Transport rates for many low molecular weight therapeutics can be augmented significantly by co-administration of permeation enhancers, such as aprotic solvents, Azone (laurocapram) and surfactants. These compounds appear to act by disrupting or altering the ordered lipid structure of the stratum corneum [9]. While low molecular weight therapeutics usually tolerate formulation with these permeation enhancers, proteins such as cytokines and lymphokines typically cannot tolerate the disruptive nature of these enhancing agents [10, 11]. Studies have suggested that liposomal formulations, which are better tolerated by protein therapeutics, can facilitate the delivery of interferon-alpha (IFN-α) *in vivo* [12] and enhance the penetration of ^{125}I-labeled rhIFN-γ into hamster, hairless mouse and human skin *in vitro* [13]. In the present study we describe methods to monitor the *in vitro* transport of biologically active rhIFN-γ across human skin and assess the potential of liposomal formulations to augment this transport.

MATERIALS AND METHODS

Reagents

Cholesterol (Ch), sodium cholate, minimal essential media (MEM), 2-[N-Morpholino]ethanesulfonic acid (MES), Tween-20, *o*-phenylenediamine and α-tocopherol were from Sigma Chemical Co. (St. Louis, MO). Thimerosal was obtained from Merck (EM Science, Gibbstown, NJ). Hanks balanced salt solution (HBSS), pH 7.4, and penicillin-streptomycin were from Gibco BRL (Grand Island, NY). Bovine serum albumin was from Intergen (Purchase, NY). Ceramide and phosphatidylcholine (PC) were from Avanti Polar Lipids (Birmingham, AL). All solvents were HPLC grade, and deionized water was filtered with a Milli-Q system (Millipore Corp., Bedford, MA) prior to use.

Formulations

E. coli-derived rhIFN-γ (Genentech, Inc., South San Francisco, CA) was formulated in 10 mM succinate (pH 5.0) containing 0.01% polysorbate 20. Lipsomes, containing 0.1% α-tocopherol, were prepared by lipid film rehydration and capillary detergent dialysis using a modified method of Schwendener [14]. Following dialysis and concentration, liposomes were stored in the dark at 4°C under N_2(g). Liposomal formulations, analyzed by dynamic light scattering (Model BI9000 AT, Brookhavens Institute, Holtsville, NY) prior to use, averaged 126 nm ± 8.1. This value increased slightly (134 nm ± 2.94) when liposomes were mixed with rhIFN-γ at a molar ratio of 4267:1. Physical mixtures of rhIFN-γ with liposomes were prepared on the day of use.

Skin and Keratinocytes

Fresh human split-thickness skin (International Institute for the Advancement of Medicine, Exton, PA) was obtained from male donors (36–62 yr.) whose death was not associated with trauma or disease. Dermatomed skin sheets, shipped overnight on ice and stored in MEM supplemented

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with 10% fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine, and penicillin-streptomycin, were used within 72 h of harvest. (Skin stored at 4°C for up to one week or at −70°C for at least six months showed comparable permeabilities to fresh skin from the same donor. Further, our data demonstrated that, despite considerable inter-donor variability, the permeabilities of abdominal, back and thigh skin obtained from the same donor were similar.) A Nikon light microscope equipped with Bioquant system IV software (R & M Biometrics, Inc., Nashville, TN) was used to determine the thickness of hematoxylin-eosin-stained skin sections (numerical averages measured for 15 skin sections and from 3 different areas of each skin section). Skin samples averaged 0.35 mm in thickness but ranged from 0.1–1.0 mm due to variable amounts of associated dermis. Cytokine transport was found to be independent of measured skin thickness (data not shown), consistent with previous studies demonstrating the stratum corneum to be the rate-limiting barrier to the transdermal passage of molecules [15].

Permeability studies were performed at 37°C for up to 24 h (typically for 5 h) using skin mounted in either Franz (Vangard International, Inc., Neptune, NJ) or Ussing-style cells (Precision Instrument Design, Tahoe City, CA). The exposed surface area for both cell types was 0.64 cm^2. While Franz cells allow for transport studies under either fully hydrated or dry conditions, the Ussing-style cells required both surfaces of the skin to be continually covered by media or solution [16]. A preincubation period of approximately one hour with HBSS was used to remove factors released from skin which interfered with rhIFN-γ assays. Donor chamber samples were taken at the beginning and termination of each experiment to account for mass balance. The entire receptor chamber volume (dermal side) was exchanged with fresh media (HBSS/0.5% BSA) at designated times for determination of rhIFN-γ concentrations. A minimum of three diffusion cells (typically four to six diffusion cells) were used for each formulation tested from the same donor. Control formulations (lacking rhIFN-γ) were tested in all experiments to correct for the release of any endogenous rhIFN-γ.

Normal adult human epidermal keratinocytes (Clonetics, San Diego, CA) treated with rhIFN-γ were used as a positive control for ICAM-1 induction. Cells were maintained in a low-calcium, serum-free medium supplemented with bovine pituitary extract and grown to subconfluency (passage 2—4). Cells were treated with 1,000 U/ml of rhIFN-γ for 16 h, trypsinized, pelleted, mounted in OCT, sectioned and stored at −70°C until use.

Calculation of Permeability

Permeability coefficients (P) were calculated using the following equation:

$$P = V(dC\ dt)/A\ C_o \quad (1)$$

where V (dC/dt) is the quantity of rhIFN-γ per unit time present in the receiver chamber, A is the surface area of the skin, and C_o is the initial concentration of the donor chamber. Alternatively, flux (J) is reported as:

$$J = P\ C_o \quad (2)$$

A log transformation was implemented in the analyses of calculated permeability coefficients. The geometric mean ± the standard error of the mean (SEM) is reported for all results.

Immunohistochemistry

Following a transport experiment, skin sections were removed from diffusion chambers, rinsed twice in sterile PBS, trimmed to include only the area exposed to the donor solution, and either placed in OCT embedding compound (Miles Laboratories Inc., Elkhart, IN) and snap frozen in liquid isopentane or incubated at 37°C in keratinocyte growth media (KGM, Clonetics) for up to 48 h and subsequently embedded in OCT, and snap frozen.

Frozen skin samples were sectioned (5 μm), air-dried on VectaBond-coated (Vector Labs Inc., Burlingame, CA) slides, fixed in methanol for 5 min. at 25°C, rinsed with PBS and blocked in 10% normal goat serum (Vector Labs Inc.) in PBS (NGS-PBS) for 30 min. Sections were incubated for 1 hour at 37°C in NGS-PBS containing either 4 μg/ml anti-human ICAM-1 antibody (Clone LB-2, Becton Dickinson Advanced Cellular Biology, San Jose, CA), an irrelevant isotype antibody or no added antibody. A Vectastain ABC-AP Kit (Vector Labs Inc.) and Vector Red II staining was used to visualize labeled ICAM-1. Sections were counter-stained with Mayer's hematoxylin, and viewed using a Leitz Aristoplan microscope equipped with a Leitz Vario Orthomat 2 microscope camera.

ELISA

A protein A-purified mouse monoclonal antibody which recognized rhIFN-γ was coated onto microtiter plates, 100 μl/well at a concentration of 10 μg/ml in PBS for 12–72 h at 2–8°C. Plates were washed (Model EL403H, Bio-Tek Instruments, Inc., Winooski, VT) with PBS, 0.05% Tween-20 and blocked with 200 μl/well of block/conjugate diluent (0.5% BSA in PBS, 0.05% Tween-20, 0.01% thimerosal, final pH 7.2 ± 0.1) for 1–6 h at 25°C. After removal of blocking

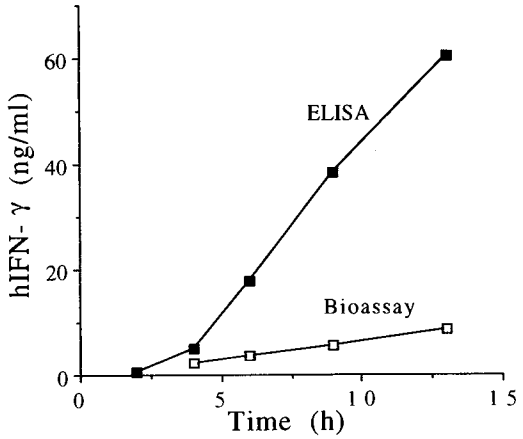

Fig. 1. A portion of rhIFN-γ transported across human split-thickness skin *in vitro* retains its biological activity. Split-thickness human skin was exposed to 0.512 mg/ml rhIFN-γ and PC/Ch (10:1) liposomes (50 mg/ml total lipid) and kept hydrated for 12 h. At selected intervals, receiver compartment concentrations were determined by either a low-level ELISA (■) or a viral-challenge cell-based bioassay (□). Data is from a representative experiment.

buffer, standards, controls, and samples (diluted in 5.0% BSA in 100 mM MES, 150 mM NaCl, 0.05% Tween-20, 0.01% thimerosal, final pH 6.1 ± 0.1) were applied (100 μl/well) and incubated at 37°C for 2 h with agitation (Mini-Orbital Shaker, Bellco Glass, Inc., Vineland, NJ). Plates were washed and an HRP-conjugated, affinity-purified, rabbit anti-hIFN-γ antibody was added. (This conjugate was prepared using an HRP-to-antibody molar ratio of 12:1 via a modification of the method described by Wilson and Nakane [17], and was diluted into block/conjugate diluent to its optimal working concentration immediately prior to addition to the plate.) Following 2 h of agitation at 25°C, plates were washed, exposed to 400 μg/ml *o*-phenylenediamine in PBS with 0.012% H_2O_2 and developed in the dark for 30 min. at 25°C. Color development was stopped by the addition of 4.5 N sulfuric acid and absorbance at 492 nm (405 nm reference)

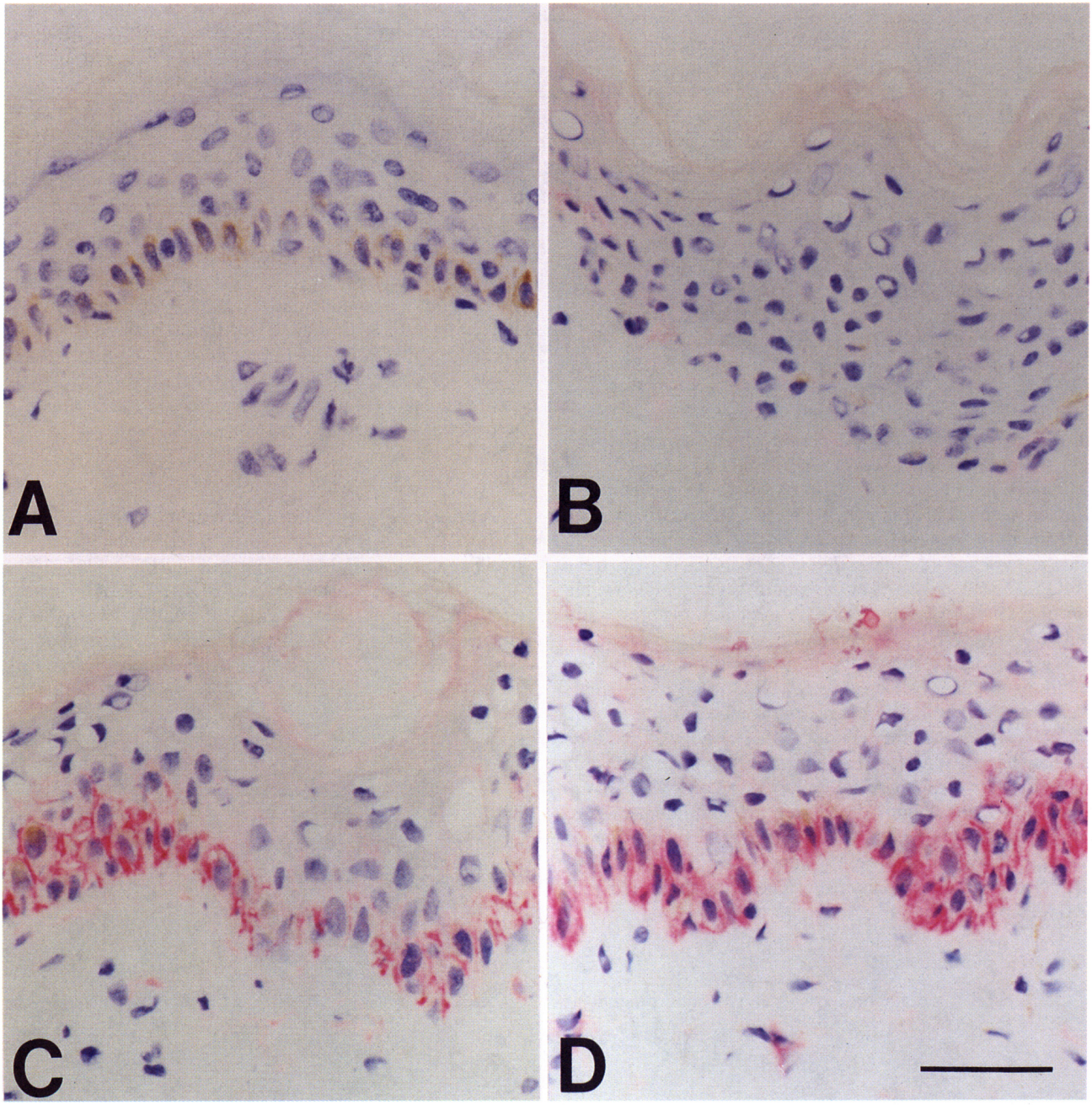

Fig. 2. Application of rhIFN-γ induces keratinocyte ICAM-1 expression in human split-thickness skin *in vitro*. Micrographs demonstrate immunolocalization of ICAM-1 in skin sections following 43 h of incubation at 37°C subsequent to a 5 h transport study with rhIFN-γ. Control tissues where the skin was not incubated (A) or was incubated with HBSS without rhIFN-γ (B) do not show any of the red labeling used to detect ICAM-1 expression. Skin tissues exposed to 0.512 mg/ml rhIFN-γ in either an aqeuous HBSS solution (C) or in an HBSS solution containing PC/Ch (10:1) liposomes at 50 mg/ml total lipid (D) demonstrate significant ICAM-1 labeling primarily at the basal layer of the epidermis. Overall, the aqueous and liposomal formulations of rhIFN-γ under hydrated conditions result in similar staining intensity and patterns. The brown stain associated with the basal cell layers in these sections is due to melanin. Bar = 50 μm.

was read (Spectra, SLT Laboratory Instruments, Tecan, USA). The concentration of rhIFN-γ in each sample was calculated relative to a standard curve generated using a nonlinear, four-parameter logistic regression analysis [18].

Bioassay

A bioassay [19], based on the ability of hIFN-γ to protect human lung carcinoma (A549) cells (CCL 185, American Type Culture Collection, Rockville, MD) from an encephalomyocarditis viral challenge was used. Quantitation of hIFN-γ antiviral activity was calibrated against a rhIFN-γ primary standard (N.I.H., Bethesda, MD) based on a 50% level of protection.

RESULTS AND DISCUSSION

Transport of rhIFN-γ across hydrated human split-thickness skin *in vitro* demonstrated an initial lag time of ~1 h and then an apparent steady-state transport over the next 12 h (Fig. 1). Mass balance recovery of rhIFN-γ from ELISA data was 99%, suggesting only a small fraction of the cytokine was lost. Low-level ELISA data, however, does not necessarily describe the amount of biologically active rhIFN-γ transported. Indeed, a viral inhibition bioassay suggested that only ~10% of the rhIFN-γ transported across the skin retained its biological activity (Fig. 1). Although the levels of rhIFN-γ reported by the bioassay were significantly lower than the ELISA, the bioassay gave reproducibly measurable amounts of rhIFN-γ at apparent steady state. It is likely that differences between the bioassay and ELISA results reflect the enzymatic degradation of rhIFN-γ during transport across the skin *in vitro*.

Intercellular adhesion molecule-1 (ICAM-1) is expressed on the surface of normal human keratinocytes 12–24 h after hIFN-γ stimulation [20]. We used this response to further assess the biological activity of transported rhIFN-γ in skin sections removed from diffusion cells following transport studies (Fig. 2). Immediately following transport no ICAM-1 expression was observed, but following subsequent culturing these same skin sections showed a weak but clearly visible induction of ICAM-1 at 12 h, and marked expression at 24 and 48 h (Fig. 2). Skin treated with either HBSS or liposomes without rhIFN-γ showed only trace ICAM-1 expression by 48 h (Fig. 2). Together, these results strongly support that at least some of the rhIFN-γ which transported across the stratum corneum of these skin samples *in vitro* was biologically active. It is important to emphasize that ICAM-1 induction only required rhIFN-γ to cross the stratum corneum and enter the epidermis, while bioassay results described rhIFN-γ transported across the full epidermis plus associated dermis.

Although ICAM-1 induction in rhIFN-γ treated skin was heterogeneous and almost exclusive to the epidermal basal layer (Fig. 2), hIFN-γ receptors in normal human skin have been demonstrated on keratinocytes in all cell layers between the basement membrane and stratum corneum [21]. There are several possible reasons for this observed pattern of ICAM-1 expression: rhIFN-γ could be sequestered at the basement membrane through interactions of the clustered basic amino acids present at its C-terminal segment which bind to glycosaminoglycans of the basement membrane [22]; additional factors required for cellular ICAM-1 expression are only present in the basal region; and/or hIFN-γ receptors are functional only in the basal region of the epidermis. It is unlikely that lipids from the liposome formulations act to impede hIFN-γ receptor activation at the superficial epidermal layers because aqueous formulations of rhIFN-γ showed a similar pattern of ICAM-1 expression.

Previous studies have demonstrated hydration of the epidermis can enhance drug transport across skin [23, 24]. We evaluated hydration as one possible role for liposome-augumented cytokine transport across human skin with the *in vitro* methods described above. Under drying conditions, the presence of added liposomes appeared to enhance the uptake of rhIFN-γ, while the same liposomal formulation had a more modest enhancing effect when skin samples were kept fully hydrated for the duration of the transport study (Table I). Consistent with the suggestion that lipids were not a significant enhancer in excess water, protein (but not lipid) concentrations dictated rhIFN-γ flux rates under fully-hydrated conditions (Table I). In a separate but similar experiment, the inclusion of ceramides in the PC/Ch liposomes

Table I. Effect of Liposome Composition on the Permeability of rhIFN-γ *in Vitro*

Formulation composition	n[a]	[Lipid][b] (mg/ml)	[rhIFN-γ] (mg/ml)	Lipid/ protein[c]	P ($\times 10^{-8}$)[d] cm/sec	J ($\times 10^{-9}$)[e] mg/sec · cm^2
Solutions allowed to dry on the skin surface						
Aqueous	6	0	0.512	N/A	1.8 ± 0.1	3.6 ± 0.2
PC/Ch (10:1)	6	50	0.512	4267	4.9 ± 1.9	9.5 ± 3.8
Solutions not allowed to dry on the skin surface						
Aqueous	53	0	0.512	N/A	2.9 ± 0.8	5.6 ± 1.5
PC/Ch (10:1)	26	10	0.512	853	3.9 ± 1.0	7.7 ± 2.0
PC/Ch (10:1)	18	50	0.512	4267	4.2 ± 1.3	8.2 ± 2.6
PC/Ch (10:1)	14	20	0.211	4267	0.15 ± 0.04	0.72 ± 0.21
PC/Ch (10:1)	5	20	0.990	853	7.9 ± 4.0	8.0 ± 4.0

[a] Number of skin samples tested, includes data collected using both Franz and Ussing-style diffusion cells, collected from eight individual donors.

[b] Total lipid comprised of both phosphatidylcholine (PC) and cholesterol (Ch).

[c] Ratios were calculated from the total moles of PC and Ch divided by moles of rhIFN-γ.

[d] Mean ± SEM under hydrated conditions at 37°C, calculated at an apparent steady state between 1 to 5 h.

[e] Mean ± SEM; calculated from permeability data and donor [rhIFN-γ].

was found to have no enhanced effect on rhIFN-γ permeability (data not shown). Also, no endogenous release of hIFN-γ from skin samples was observed by ELISA over the time course of these experiments.

Further studies to evaluate the effects of hydration on rhIFN-γ transport were performed by extending transport experiments to a total of 24 h while the donor side was allowed to dry (Fig. 3A). A striking decrease of rhIFN-γ transport was observed several hours after the surface of the skin appeared dry. Increased permeability at early times through skin where the donor was allowed to evaporate may have been due to an increase in protein concentration as the donor solution dried, providing an increase in C_o compared to that in the fully-hydrated sample. To further test this, slightly different conditions were established where skin exposed to rhIFN-γ formulated with or without liposomes (50 mg/ml total lipid) was allowed to dry and then was repeatedly dosed

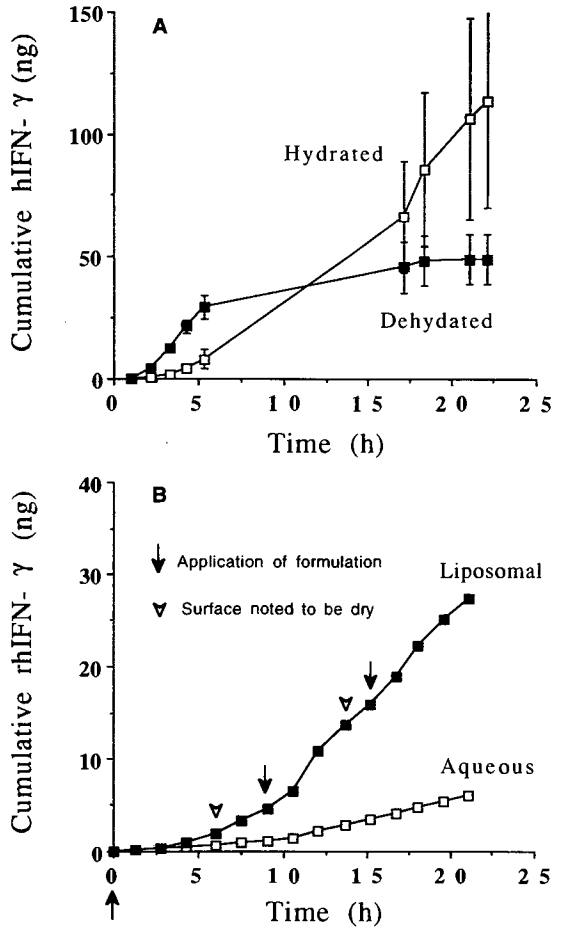

Fig. 3. Hydration affects rhIFN-γ permeability across split-thickness skin *in vitro*. A) Skin was mounted in either Franz cells (n=3) with 50 μl or in Ussing-style cells (n=5) with 1 ml of 0.512 mg/ml with 50 mg/ml of liposomes applied to the external skin surface for both cell types. After approximately 5 h the surface of the skin appeared dry in the Franz cells (dehydrated). Ussing-style cells remained fully hydrated for the duration of the experiment. B) Skin samples were exposed to repeated applications (arrows) of rhIFN-γ formulations (50 μl) containing 0.512 mg/ml of the cytokine with 50 mg/ml total lipids (Liposomal) or no added lipid (Aqueous). Between applications the skin surface was allowed to dehydrate (arrowheads signify times of observed dryness).

with the same formulation (Fig. 3B). Here, the liposomal formulation provided a striking enhancement of rhIFN-γ transport suggesting that the process of drying (and thus concentration) of the formulation may act to increase the driving force for cytokine penetration into the skin to a greater extent for liposomal formulations. Although there is currently no data to evaluate the fate of the lipid from these formulations, such information may ultimately be of interest since stratum corneum lipid content can affect transport water across skin *in vitro* [25, 26] and exogenous lipid introduction can change the physical properties of the epidermis [27]. Indeed, concerns about dried lipids on the skin surface as an additional barrier for cytokine transport has already been discussed [13].

Previous studies have shown the benefit of topically administered liposome-encapsulated drugs [28] including interferons [12, 13]. In our studies we used a physical mixture, i.e. a solution prepared by the combination of preformed liposomes with a stabilized formulation of rhIFN-γ, using liposomes of similar lipid composition to those used in these previous studies. The use of such a physical mixture, rather than encapsulation, is supported by previous studies which suggest that intact liposomes are not required for enhanced drug transport [29] nor do intact liposomes appear capable of penetrating deeper than the cornified layer of human skin [30]. Although the presence of liposomes only modestly enhanced rhIFN-γ transport across fully-hydrated skin, the application of liposomes to skin under conditions where evaporation of the formulation can occur, leads to an increase in cytokine transport. From our studies, we suggest one putative mechanism for the enhanced transport observed with liposomal formulations of biologically active rhIFN-γ across split-thickness skin *in vitro* may involve an increased hydration of the stratum corneum beyond what is observed with application of an aqueous formulation.

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REFERENCES

1. J.M. Hanifin, L.C. Schneider, D.Y.M. Leung, C.N. Ellis, H.S. Jaffe, A.E. Izu, L.R. Bucalo, L.R. Hirabayashi, S.J. Tofte, G. Cantu Gonzales, H. Milgrom, M. Boguniewicz, K.D. Cooper. Recombinant interferon gamma therapy for atopic dermatitis. *J. Am. Acad. Dermatol.* 28:189–197 (1993).
2. R.D. Granstein, A. Rook, T.J. Flotte, A. Haas, R.L. Gallo, H.S. Jaffe, E.P. Amento. A controlled trial of intralesional recombinant interferon-γ in the treatment of keloidal scarring. *Arch. Dermatol.* 126:1295–1302 (1990).
3. U. Reinhold, S. Kukel, B. Goeden, U. Neumann, H.W. Kreysel. Functional characterization of skin-infiltrating lymphocytes in atopic dermatitis. *Clin. Exp. Immunol.* 86:444–448 (1991).
4. S. Kakumu, T. Ishikawa, M. Mizokami, E. Orido, K. Yoshioka, T. Wakita, M. Yamamoto. Treatment with human gamma inter-

feron of chronic hepatitis B: Comparative study with alpha interferon. *J. Med. Virology* 35:32–37 (1991).
5. P.G. O'Connell, L.H. Gerber, J.J. Digiovanna, G.L. Peck. Arthritis in patients with psoriasis treated with gamma-interferon. *J. Rheumatol.* 19:80–82 (1992).
6. P.M. Elias, D.S. Friend. The permeability barrier in mammalian epidermis. *J. Cell Biol.* 65:180–191 (1975).
7. P.M. Elias, J. Goerke, D.S. Friend. Mammalian epidermal barrier layer lipids: composition and influence on structure. *J. Invest. Dermatol.* 69:535–546 (1977).
8. K.V. Roskos, R.H. Guy. Assessment of skin barrier function using transepidermal water loss: effect of age. *Pharm. Res.* 6:949–953 (1989).
9. A.C. Williams, B.W. Barry. Skin Absorption Enhancers. *Crit. Rev. in Therap. Drug Carrier Syst.* 9:305–355 (1992).
10. Y-C.J. Wang, M.A. Hanson. Parenteral formulations of proteins and peptides: stability and stabilizers. *J. Parenteral Sci. Technol.* 42:S3–S26 (1988).
11. M.C. Manning, K. Patel, R.T. Borchardt. Stability of protein pharmaceutics. *Pharm. Res.* 6:903–918 (1989).
12. N. Weiner, N. Williams, G. Birch, C. Ramachandran, C. Shipman Jr., G. Flynn. Topical delivery of liposomally encapsulated α-interferon in a cutaneous herpes guinea pig model. *Antimicro. Agents Chemother.* 33:1217–1221 (1989).
13. J. du Pleiss, K. Egbaria, C. Ramachandran, N. Weiner. Topical delivery of liposomally encapsulated gamma-interferon. *Antiviral Res.* 18:259–265 (1992).
14. R.A. Schwendener. The preparation of large volumes of homogeneous, sterile liposomes containing various lipophilic cytostatic drugs by the use of a capillary dialyzer. *Cancer Drug Delivery* 3:123–129 (1986).
15. M.L. Francoeur, R.O. Potts. The influence of stratum corneum morphology on water permeability. *J. Invest. Dermatol.* 96:495–499 (1991).
16. G.M. Grass, S.A. Sweetana. *In vitro* measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.* 5:372–376 (1988).
17. M.B. Wilson, P.K. Nakane. Recent developments in the periodate method of conjugating horseradish peroxidase (HPO) to antibodies, in "Immunofluorescence and related staining techniques" W. Knapp, K. Holubar, G. Wicks, eds. Elsevier/North-Holland Biomedical Press, pp. 215–224 (1987).
18. D. Marquardt. An algorithm for least-square estimation of nonlinear parameters. *J. Soc. Indust. Appl. Math* 11:431–441 (1963).
19. N.H. McManus. Microtiter assay for interferon: microspectrophotometric quantitation of cytopathic effect. *Appl. Environ. Microbiol.* 31:35 (1976).
20. A.P. Winiski, C.A. Foster. ICAM-1 expression in a spontaneously transformed human keratinocyte cell line: characterization by a simple cell-ELISA assay. *J. Invest. Dermatol.* 99:48–52 (1992).
21. A. Scheynius, J. Fransson, C. Johansson, H. Hammar, B. Baker, L. Fry, H. Valdimarsson. Expression of interferon-gamma receptors in normal and psoriatic skin. *J. Invest. Dermatol.* 98:255–258 (1992).
22. H. Lortat-Jacob, J.-A. Grimaud. Interferon-γ binds to heparin sulfate by a cluster of amino acids located in the C-terminal part of the molecule. *FEBS LETTERS* 280:152–154 (1991).
23. S-K. Chang, J.E. Riviere. Effect of humidity and occlusion on the percutaeous absorption of parathion *in vitro*. *Pharm. Res.* 10:152–155 (1993).
24. D.A.W. Bucks, H.I. Maibach, R.H. Guy. Occlusion does not uniformly enhance penetration *in vivo*, in "Percutaneous absorption" R.L. Bronough, H.I. Maibach, eds. Marcel Dekker, New York, p. 87, (1989).
25. Y. Morimoto, T. Hatanaka, K. Sugibayashi, H. Omiya. Prediction of skin permeability of drugs: comparison of human and hairless rat skin. *J. Pharm. Pharmacol.* 44:634–639 (1992).
26. K. Harada, T. Murakami, N. Yata, S. Yamamoto. Role of intercellular lipids in stratum corneum in the percutaneous permeation of drugs. *J. Clin. Invest.* 99:278–282 (1992).
27. B. Ongpipattanakul, R.R. Burnette, R.O. Potts, M.L. Francoeur. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharm. Res.* 8:350–354 (1991).
28. V. Masini, F. Bonte, A. Meybeck, J. Wepierre. Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. *J. Pharm. Sci.* 82:17–21 (1993).
29. C. Michel, T. Purmann, E. Mentrup, E. Seiller, J. Kreuter. Effect of liposomes on percutaneous penetration of lipophilic materials. *Int. J. Pharm.* 84:93–105 (1992).
30. J. Lasch, R. Laub, W. Wohlrab. How deep do intact liposomes penetrate into human skin? *J. Contr. Rel.* 18:55–58 (1991).