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Invited article

Recent Observations Regarding Interferon, Keratinocytes and Lymphocytes *In vitro* and *In vivo*

Brian J. Nickoloff

The purpose of this presentation is 3 fold and includes the past, present, and future:

1. To review the concept of skin-associated lymphoid tissue (SALT)
2. To summarize the experimental work related to lymphocyte-keratinocyte reactions *in vitro*
3. To briefly outline interferon related therapeutic implications for dermatologists

In the past few years, several review articles concerning the immunobiology of the skin have appeared (1-3). The initial skin biologists were concerned with understanding how the epidermis could influence and interact with lymphocytes. Analogies between thymic epithelium and epidermis were made and keratinocytes were found to be able to induce terminal deoxynucleotidyl transferase on T lymphocytes (4). Next, Chu et al. (5) demonstrated that keratinocytes peroduced a thymopoietin-like molecule which could also induce T-lymphocyte differentiation. Most recently, keratinocytes have been found to produce an Interleukin-1 factor (Epidermal Thymocyte Activating Factor, ETAF) which can augment the proliferation of partially stimulated lymphocytes (6, 7). With the discovery that epidermal Langerhans cells could function as specialized antigen presenting cells to T lymphocytes, there could be little doubt that the skin was more than a passive target for various immunologically-mediated insults, but rather was an active participant in the initiation and propagation of such events (8). Streilein attempted to integrate all these results into the concept of a skin-associated lymphoid tissue (SALT) hypothesis in which he proposed that the immunological microenvironmental endowment of skin served an immunosurveillance function protecting against infectious and neoplastic assaults (2).

The work done at Stanford University during the past 3 years has attempted to extend the concept of SALT by focusing not on how keratinocytes influence lymphocytes but on elucidating how lymphocytes may influence keratinocyte growth and differentiation. Our approach to establishing the proposition of reciprocal keratinocyte-lymphocyte interactions *in vivo*, was to examine the effects of lymphocyte produced factors such as interferons (IFNs) on cultured human keratinocytes.

While interferons were originally described as antiviral agents (9), they have been found to affect a wide range of other cellular biologic functions (10). Interferons are currently classified into two different groups; types I and II. Type I IFNs include alpha-interferon, derived primarily from leukocytes and beta-interferon derived from fibroblasts and epithelial cells. Gamma interferon is a Type II interferon which is produced by activated T lymphocytes.

Utilizing recombinant alpha, beta and gamma interferons both alone and in combination, we have made the following observations:

1. Recombinant gamma interferon (r-IFN-γ) induces the synthesis and expression of HLA-DR and other triton soluble proteins on cultured human keratinocytes (11-13).
2. R-IFN-α and r-IFN-γ inhibit the growth of keratinocytes in a concentration dependent

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Department of Pathology, University of Michigan Medical Center, M 4232 Medical Science I, Ann Arbor, Michigan 48109-0602, U.S.A..

fashion (14).

3. R-IFN-γ induces ultrastructural changes in keratinocytes suggesting an influence on differentiation (15).

4. R-IFN-α, β, γ possess antiproliferative and immunomodulatory activity on a cutaneous squamous cell carcinoma *in vitro* with both additive and synergistic effects seen combining type I and II interferons (16).

These results have been recently confirmed by others (17–19). Most recently, we have undertaken studies to examine the possible immunobiological consequences of keratinocyte HLA-DR expression (20).

To investigate the significance of HLA-DR expression by cultured keratinocytes, we initially asked whether this class II antigen would be recognized by allogeneic peripheral blood mononuclear leukocytes (PBMLs). Compared to HLA-DR positive Langerhans cells (LCs), the DR+ keratinocytes were relatively weak stimulators of resting T lymphocyte proliferation (21). While the allogeneic PBMLs apparently recognized allo-antigen on the keratinocytes by increasing in size, and RNA synthesis, initial activation was not followed by proliferation or mitogenesis as determined by ^{3}H-thymidine incorporation or cell number (ibid). A partial explanation for this "block" in T lymphocyte proliferation may have resulted from the increased production by the keratinocytes of PGE2 by gamma interferon, although other keratinocyte derived inhibitory factors may be important in this reaction. These results also may signify that only dendritic HLA-DR positive cells such as LCs are capable of promoting resting T lymphocyte proliferation (22). When the resting T lymphocytes are indubated with HLA-DR positive keratinocytes in the presence of interleukin-2 (IL-2) a much greater proliferative response is observed. Thus *in vivo*, after initial activation by LCs, the PBMLs may continue to interact in biologically important ways with HLA-DR positive keratinocytes.

Since HLA-DR expression on keratinocytes is a common feature of 50 or more different dermatoses, it is important that more work be done on characterizing lymphocyte-keratinocyte interactions (23). D. Krueger and coworkers have suggested that HLA-DR positive keratinocytes may influence LC migration into the skin *in vivo* (24). Also, it has been suggested that HLA-DR positive keratinocytes may be targeted for T-cell mediated cytotoxicity in various lichenoid tissue reactions (25). Another important area of research in studies of the role of interferons in dermatology involves the antiviral rather than the immunomodulatory or antiproliferative activity of interferons.

By exposing cultured human keratinocytes to herpes simplex virus (HSV) *in vitro*, it was previously observed that interferon production could be detected (26). Using highly specific neutralizing antibodies against alpha, beta and gamma interferon, it has been shown that keratinocytes produce predominantly beta interferon after HSV infection (27). While all three types of interferon have been found in HSV vesicle fluid, it was the amount of gamma interferon which was found to correlate with the recurrence rate (28). Another intriguing recent observation regarding HSV infections in that a non PGE2 soluble factor derived from epidermal cells co-incubated with HSV antigen was isolated after UV-B exposure which inhibited various immunological functions of lymphocytes (29). These authors suggest that the production of this inhibitory factor after intensive sun exposure may be responsible for the associated occurrence of recurrent herpes labialis.

Finally, what are the possible therapeutic implications of interferons for dermatology? With the advent of recombinant biotechnology the production of highly purified interferons are widely available for trials. To date, dermatologists have taken advantage of the antiviral, immunomodulatory and antiproliferative activities of interferons by utilizing them in the following diseases (see appendix):

I. Herpes Simplex (30–32)
II. Verruca Plantaris (33, 34)
III. Condyloma Accuminata (33, 35, 36)
IV. Epidermodysplasia Verruciformis (37)
V. Bowenoid Papulosis (38)
VI. Laryngeal Papillomatosis (39)
VII. Leprosy (40)
VIII. Psoriasis (41, 42)
IX. Actinic Keratosis (43)
X. Melanoma (44–46)
XI. Mycosis Fungoides (47)

It appears that intralesional or systemic administration is superior to topical application of interferon for these diseases and that interferons are more efficacious as anti-viral agents than antiproliferative agents at this time. I believe in the future, therapeutic protocols will involve combinations of type I and II interferons and addition of other factors such as IL-2, tumor necrosis factor, etc. Topical preparations combining interferons with surfactant-like molecules to increase epidermal permeation of the interferons, such as Nonoxynol 9 may be more efficacious than interferons alone (48). Much has been learned in the past several years regarding lymphocyte-keratinocyte interactions and many additional therapeutic trials using interferons have been published since the review article by Yancey and Smith in 1980 (49). Considerable work remains ahead both in the laboratory and in the development of new therapeutic strategies utilizing interferon in dermatology (50, 51).

—Appendix—

Summary of Clinical Trials Using Interferon in Dermatology

Interferons and Dermatology

I. Herpes Infections
II. Verruca Plantaris
III. Condyloma Accuminata
IV. Epidermodysplasia Verruciformis
V. Bowenoid Papulosis
VI. Laryngeal Papillomatosis
VII. Leprosy
VIII. Psoriasis
IX. Actinic Keratosis
X. Melanoma
XI. Mycosis Fungoides

I. Herpes Infections

	Disease	Type Interferon*	Results	Investigator
A.	Recurrent herpes labialis after surgery trigeminal neuralgia	Non-recombinant alpha	Reduced viral shedding from 42–9% and reactivation from 83–47%	Pazin et al., 1979
B.	Herpes zoster in cancer patients	Non-recombinant alpha	Limited cutaneous spread, progression in primary dermatome and visceral complications	Merigan et al., 1978
C.	Varicella in childhood malignancy	Non-recombinant alpha	Decreased life-threatening dissemination	Arvin et al., 1978
D.	Cutaneous herpes simplex virus	Non-recombinant alpha	Complete and rapid healing of skin lesions	Shalev et al., 1984

*Unless otherwise specified, all interferons were administered intramuscularly for systemic effects.

II. Human Papillomavirus Infections

	Disease	Type Interferon	Results	Investigator
A.	Verruca Plantaris (HPV 1, 2)	Recombinant alpha-2 Intralesional	No benefit	Vance et al., 1986
B.	Verruca Vulgaris	Non-recombinant beta Intralesional	Good response in 81% versus 17% placebo	Niimura, 1983
C.	Condylomata Accuminata (HPV 6, 11)	Recombinant alpha-2 Intralesional	Complete clearing in 53% versus 14% placebo	Vance et al., 1986
		Recombinant alpha-2c	Objective response in 72%	Gross et al., 1986
		Non-recombinant beta	Good response in 82% versus 9% placebo	Schonfeld et al., 1984

D.	Bowenoid Papulosis (HPV 16)	Recombinant alpha-2c	Complete response in 1 patient and partial clearing in 2 others	Gross et al., 1986
E.	Epidermodysplasia Verruciformis (HPV 3, 5, 8)	Non-recombinant alpha, systemic and Intralesional	75–80% of patients with regression	Androphy et al., 1983
F.	Juvenille Laryngeal Papillomatosis (HOP 11)	Non-recombinant alpha	Tumor control in all cases	Haylund et al., 1981

III. Leprosy

	Disease	Type Interferon	Results	Investigator
A.	Lepromatous Leprosy	Recombinant gamma intralesional	Augmentation of Monocyte H_2O_2 release	Nathan et al., 1986

	Disease	Type Interferon	Results	Investigator
IV.	Psoriasis Vulgaris	Recombinant gamma	Slight benefit at highest dose range	Morhenn et al., 1987
		Recombinant alpha	Exacerbation	Quesada et al., 1986

	Disease	Type Interferon	Results	Investigator
V.	Actinic Keratosis	Recombinant alpha cleared after 6 injections	14 of 15 lesions	Edwards et al., 1986

VI. Melanoma

	Disease	Type Interferon	Results	Investigator
A.	Metastatic Melanoma	Non-recombinant alpha plus cimetidine	5 of 20 patients with complete regression of cutaneous/ subcutaneous lesions	Odgren et al., 1983
B.	Disseminated Melanoma	Recombinant alpha-A	20% objective partial regression	Creagan et al., 1984
C.	Metastatic Melanoma	Recombinant alpha-2	25% objective partial or complete response	Kirkwood et al., 1985

VII. Cutaneous T-cell Lymphoma

	Disease	Type Interferon	Results	Investigator
A.	Mycosis Fungoides and Sezary Syndrome	Recombinant alpha-A	9 of 20 patients with objective partial remission	Bunn et al., 1984

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