Effects of Isotretinoin on the Neutrophil Chemotaxis in Cystic Acne

To the Editor:

I have read with interest the article of Norris et al. "Isotretinoin produces significant inhibition of monocyte and neutrophil chemotaxis in vivo in patients with cystic acne" in the July 1987 Journal of Investigative Dermatology. Their in vivo results are similar to our unpublished in vivo results for a larger group (Table I) of patients. We studied 25 male patients receiving Isotretinoin at a dosage of 0.8 mg/Kg body weight for a period of 15 d. For in vivo chemotaxis, a modification of the method of Breathnach et al was used [1].

However, their in vitro results, though obtained with a method similar to ours [2], are completely different from those we published. In their studies the pretreatment neutrophil chemotaxis from acne patients was enhanced and did not diminish significantly with treatment. In our group the pretreatment values were not significantly different from normal, but did decrease significantly with treatment.

We could explain this difference if their 8 patients were having flare-ups of acne after 1 month without treatment, because chemotaxis of neutrophils is as high in acute neutrophilic dermatosis as it is in acute pustular psoriasis [3].

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Table I. In vivo chemotaxis values for patients with cystic acne and normal controls.

Normal Controls	Cystic Acne	
	before treatment mean ∓ S.D. (range)[a]	after treatment mean ∓ S.D. (range)[a]
132 ∓ 14 (55–168)	146 ∓ 20 (44–187)	76 ∓ 7.8[b] (62–91)

[a] all activities are expressed as PMNL × 10^6 per square cm
[b] $p < 0.01$ statistically different.

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REPLY

Thank you for providing your data confirming our finding that oral isotretinoin significantly inhibits neutrophil chemotaxis in vivo as reported in this journal [1]. You also refer to an earlier article [2] in which you demonstrated a 15% decrease in the mean chemotactic response of eight patients receiving isotretinoin and imply that the decrease accounts for the biologic effects observed in vivo. You also state that apparent differences between our results might be based on "flare-up" of acne in our patients.

Using the in vivo chemotaxis assay that you employed in your studies, you found approximately a 50% decrease in neutrophil migration in patients receiving isotretinoin. We reported 98% inhibition of neutrophil and monocyte chemotaxis following isotretinoin treatment, which is testimony to the sensitivity and low background migration obtained using our in vivo technique, originally reported in 1979 [3].

However, we are concerned with the 15% inhibition in chemotaxis in vitro which you reported in Ref 2. The inhibition was restricted to only four of the eight patients, with four showing almost no inhibition of response. In addition, the leading front technique is *not* necessarily the best choice for comparison with your in vivo results. Analysis of total cell numbers which migrated a defined distance would be more analogous to your measurements in vivo. The 15% reduction which you report in vitro cannot begin to explain the 50% reduction which you reported in vivo (nor the 98% inhibition which we reported). Our patients were not experiencing disease exacerbations after one month off antibiotics. Although their neutrophil responses were decreased below pretreatment values after one month of treatment, they were not lower than normal controls.

On a conceptual level, there are additional problems with your implication that one must be able to show drug effects on the chemotactic response in vitro to mimic the in vivo inhibition of cell mobilization. If isotretinoin merely interfered with neutrophil migration to a chemotactic gradient, we might expect to see a population of neutrophils outside the dermal vessels moving toward an epicutaneous attractant in isotretinoin-treated patients. This we did *not* observe in our assay system. We suspect that isotretinoin effects on endothelial/neutrophil interactions may be an important site of action. We have reported preliminary information on inhibition of neutrophil migration through endothelial cell-coated filters by isotretinoin [4].

Finally, the reason for your highly variable results with neutrophil chemotaxis in vitro using neutrophils from isotretinoin-treated patients (4 with inhibition, 4 without inhibition) may be due to the highly reversible effects of the active metabolites of isotretinoin, which we have observed in recent experiments using 4-keto and 4-hydroxy isotretinoin [5].

In the near future, we hope to publish our results on the inhibitory effect of these metabolites on neutrophil migration, using standard chemotactic assays and assays using barrier filters coated with endothelial monolayers. It appears that the profound anti-inflammatory effects of isotretinoin are more complex than implied in your letter.

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Class II MHC Antigen Expression by Keratinocytes Results From Lymphoepidermal Interactions

To the Editor:

I would like to present some additional information and comments related to the results and conclusions of the paper by N. E. Wikner et al [1].

My colleagues and I, like the authors of this paper, hypothesized that the signal for HLA-DR expression by keratinocytes in different inflammatory dermatoses comes from the infiltrating skin lymphocytes. To examine this hypothesis, we used a system that allows the in vitro study of different aspects of lymphoepidermal interactons [2]. In this mixed skin cell-lymphocyte culture reaction (MSLR), dispersed epidermal cells (EC) are cocultured with purified peripheral blood lymphocytes (PBL). To determine whether during lymphoepidermal interactions in vitro a factor(s) inducing HLA-DR expression on keratinocytes may be generated, we cocultured PBL with either allogeneic, autologous, or PPD-pulsed autologous EC. The supernatants from these different types of MSLR as well as from controls were then harvested at different times and used to treat separately cultured human EC. As shown in Fig 1*A*, the supernatants from allogeneic and PPD-pulsed autologous MSLR contained the factor(s) that stimulated HLA-DR antigen synthesis and expression by 30–40% of cultured EC. Kinetic analysis revealed a production rate maximum between 72–96 h of lymphoepidermal coculturing.

The supernatants from these 2 types of MSLR demonstrated in classic interferon (IFN) assay, the ability to inhibit a cytopathogenic effect of vesicular stomatitis virus on human amnion WISH cells. Furthermore, the activity of these supernatants was susceptible to low pH (pH. 2), high temperature (56°C), and could also be inhibited by anti-γ-IFN but not by anti-α-IFN antibody pretreatment (Fig 1*B*). Thus, as documented by Wikner et al [1], we found that γ-IFN is responsible for HLA-DR expression by keratinocytes.

In the experiments described by Wikner et al [1], the lack of HLA-DR expression by keratinocytes (5–7 days-old cultures) cocultured with PBL (without concanavalin A) was probably due to the absence of lymphocyte stimulation. As we reported [3], freshly isolated EC vigorously stimulate the allogeneic PBL in MSLR, but during EC culture their capacity to induce PBL responses progressively diminishes. This phenomenon is due to the disappearance of functionally active Langerhans cells.

Recent data demonstrated that HLA-DR (+) keratinocytes were neither more potent stimulators of allogeneic PBL [4] nor did they induce higher mitogen-driven T-cell proliferation [5] than HLA-DR (−) keratinocytes. In view of the above data, it seems that in the case of human keratinocytes, the induction of HLA-DR antigen is not an essential primary event in triggering an immune response but is, rather, secondary to increased levels of γ-IFN found as part of a generalized release of T lymphocyte-derived lymphokines during lymphoepidermal interactions.

Furthermore, the possibility that secondary increases in class II MHC antigen expression could aggravate the autodestructive process [i.e., HLA-DR (+) keratinocytes may well be the target for the activated cytotoxic T lymphocytes] cannot be ignored and the potential immunologic role of keratinocytes warrants further experimental consideration.

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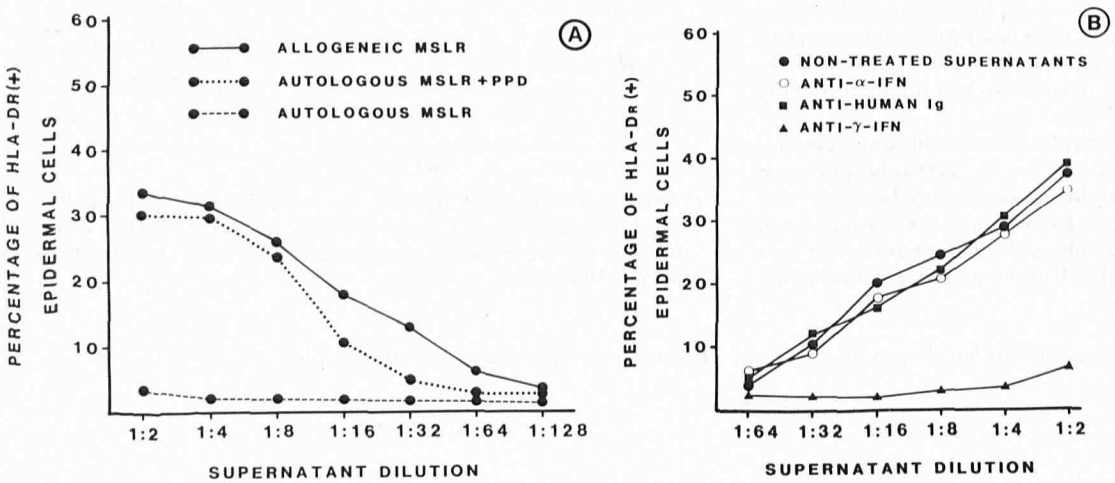

Figure 1. Percentage of HLA-DR positive EC after 96-h incubation with supernatants provided from different MSLR. *A*, allogeneic, autologous, or PPD-pulsed autologous MSLR supernatants. *B*, supernatant from PPD-pulsed autologous MSLR was preincubated (2 h. 37°C) with either anti-α-IFN, anti-γ-IFN, or antihuman Ig antibodies before their addition to the EC cultures. The means of triplicate samples are given.