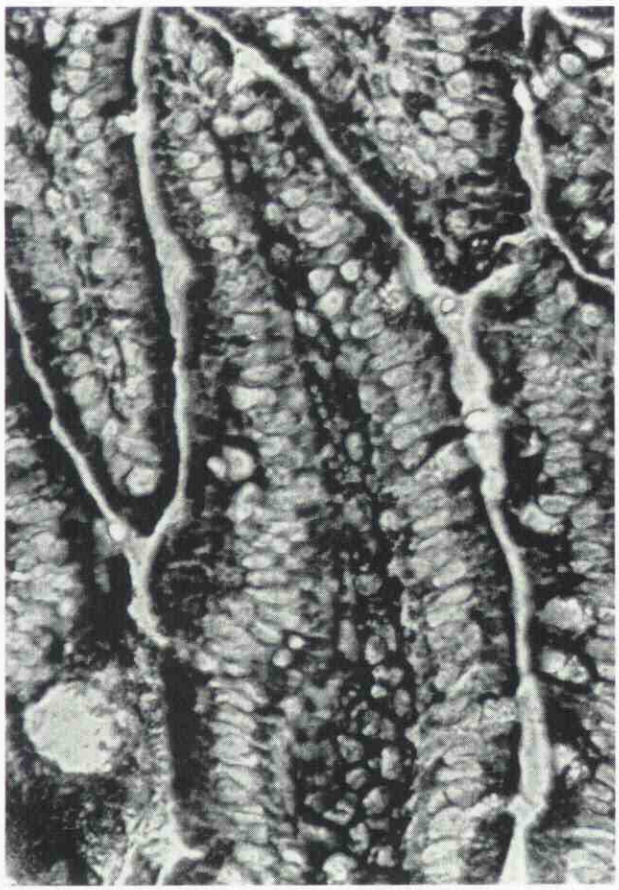

Figure 2. Indirect immunoperoxydase staining for Ia on cryostat section of normal mouse duodeno-jejunum. (Magnification ×40.)

REPLY

We appreciate the comments of Kaiserlian and Nicolas regarding the constitutive expression of class II antigens (Ia) by murine enterocytes. In our study [1], Ia expression by resting murine enterocytes was not detected by indirect immunofluorescent staining using monoclonal antibodies (MoAb) MK.D6 and 14-4-4S. These MoAb possess restricted antigen specificity, and most likely bind single epitopes expressed on murine I-A^d and I-E molecules, respectively. In comparison, Kaiserlian and Nicolas present convincing evidence (their Fig 2) that murine enterocytes constitutively express Ia, when analyzed with a MoAb, CD311, that has broader antigen specificity for framework determinants associated with the alpha and beta chains of both I-A and I-E molecules. Given this data, we agree with the conclusions of Kaiserlian and Nicolas, that different MoAb may vary in their ability to detect poorly accessible determinants or weakly expressed antigens in sections of frozen tissue. A brief review of the literature tends to support the potential for reporting discrepancies on constitutive class II antigen expression by enterocytes based on the antibodies employed for detection [2–5]. It appears that reports of constitutive class II antigen expression by enterocytes correlates with the use of MoAb with broader specificity.

Kaiserlian and Nicolas also point out the fact that Ia^+ cells may be more readily induced to express higher amounts of Ia when treated with gamma-interferon (IFNγ). This would help explain the differences in the dose response to IFNγ of keratinocytes and enterocytes we observed [1]. If, as shown by Kaiserlian and Nicolas, enterocytes constitutively express a low amount of Ia we agree that they would be more sensitive to IFNγ-induced Ia expression than Ia^- keratinocytes. Regarding our study, it would be of interest to determine whether low-dose IFNγ-treated keratinocytes are induced to express detectable levels of Ia using a more sensitive MoAb, such as CD311. A more critical examination and comparison of the activities of the different reagents employed in various studies will provide a clearer understanding of the biologic systems under investigation.

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Antigen-Presenting Cells in the Induction of Contact Hypersensitivity in Mice: Evidence That Langerhans Cells Are Sufficient But Not Required

To the Editor:

We read with great interest the article by Streilein [1] in the October 1989 issue of the Journal. While concurring with his central thesis, that epidermal Langerhans cells (LC) are not the only skin cells capable of presenting contact sensitizers, we take issue with several of his assumptions and conclusions.

First, Dr. Streilein asserts that low-dose ultraviolet B (UVB) irradiation *depletes LC* from mouse epidermis. This assertion misinterprets results of previous investigations [2,3]. UVB in fluences less than 10^3 J/m^2 does diminish the density of LC as measured by surface ATPase activity [2,3] or Ia expression [3]. These phenotypic alterations, however, do not equate with physical absence of LC since "substantial numbers of virtually unaltered LC (as judged by electron microscopy) were present at a time when their surface markers were no longer identifiable" [3]. Further, recent evidence indicates that loss of LC surface markers need not be associated with loss, but rather alteration, of LC function: 1) when placed in culture, LC lose surface ATPase activity but gain Ia expression with concur-

rent changes in their immunogenic capacity [4], and 2) we have shown low-dose UVB to convert LC from inducers to down-regulators of contact hypersensitivity (CH) [5].

Second, Dr. Streilein states that cellophane tape stripping of mouse epidermis *completely removes LC.* His own original work refutes this; repeated cellophane stripping (15 times) led to persistence of (albeit few) $ATPase^+$ or Ia^+ dendritic cells adjacent to hair follicles [6]. Since very small numbers of LC (as few as 10 according to Dr. Streilein's studies) are required to generate delayed-type hypersensitivity responses in vivo [7,8] including CH [5], it is possible that residual LC contribute to, perhaps are largely responsible for, the sensitization generated after DNFB-painting of tape-stripped mice.

Third, Dr. Streilein treats the phenomenon of LC depletion in tape-stripped mice as if it occurs in a vacuum. UVB radiation exerts disparate influences on different skin components; it is likely that tape stripping or other exogenous insults for that matter also lead to multiple inflammatory, even immunologic, consequences. To begin with, depletion of LC following tape stripping is transient; substantial numbers of (presumably migrant) LC appear within 24 h after stripping [6]. Does tape stripping alter the function of residual or of immigrant LC? Perhaps it up-regulates their immunogenicity? What happens to residual keratinocytes during this process? Is their cytokine-producing capacity impaired? Are $Thy\text{-}1^+$ dendritic epidermal cells ($Thy\text{-}1^+$DEC) also depleted?

These issues are critical in the context of Dr. Streilein's premise, that findings derived from the tape-stripping model provide insight into the genesis of low-dose UVB-induced suppression of CH. Our view is that these two models are disparate experimental systems; whereas LC are actually lost after tape stripping, these cells remain but with altered properties following low-dose UVB exposure. In light of this fundamental difference and other arguments presented herein, we question the validity of his premise.

Fourth, Dr. Streilein favors "sparing" of the tolerogenic cellular source to account for UVB susceptibility in relevant mouse strains. In fact, our studies (admittedly limited to UVB susceptible mice) lend support to this notion; low-dose UVB exerted no effect on the inherent suppressive signal produced by $Thy\text{-}1^+$DEC in CH [5]. On the other hand, identical phototreatment of LC led to the conversion of LC function from induction to down-regulation of CH [5]. On this basis, we offer an alternative hypothesis: low-dose UVB irradiation confers upon LC the capacity to initiate immunosuppression; once evoked, this attribute remains dominant and is manifest in vivo in susceptible, but not in resistant, strains. We do agree with Dr. Streilein's final assessment, that the definitive experiments to explain UVB susceptibility have yet to be performed.

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REPLY

The critical comments of Dr. Ponciano D. Cruz concerning our recent article in JID (Streilein JW: Antigen-presenting cells in the induction of contact hypersensitivity in mice: Evidence that Langerhans cells are sufficient but not required. J Invest Dermatol 93:443–448, October 1989) are welcomed in that they reveal that someone "out there" actually cares about the issues raised.

The premise as well as the conclusion drawn from the experiments presented in that paper are asserted in the title. Langerhans cells may be capable of functioning as antigen-presenting cells in the induction of contact hypersensitivity, but that are certainly not essential to that induction. Two of the experimental strategies used, irradiation with low-dose UVB and tape stripping, were selected because both have been demonstrated to deplete epidermis of normal-appearing and normally functioning Langerhans cells.

Dr. Cruz objects because he believes that the article states that UVB radiation and tape stripping "completely remove all Langerhans cells from treated epidermis." When we collaborated in Dallas, Dr. Bergstresser (Dr. Cruz' mentor) and I agonized at length over the semantic issues involved in choosing appropriate words to describe succinctly the effects of these treatments on epidermal Langerhans cells (LC). We tried very carefully to use words, such as "deficient" and "deplete," which convey the idea that neither treatment completely removes all traces of LC. Often we inserted the adjective "functional" (modifying depletion or deficiency) to underscore this fact. However, once this qualification has been made in a manuscript, it seems unnecessary to add the qualifier each time the point is raised. To that end, the Introduction to the article in question uses the terms "depleted," "LC-deficient," and "severely depletes" to describe the quantitative and qualitative effects of tape stripping and of UVB treatment, and to imply that some LC, in one form or another, still remain after these procedures. Anyone who is familiar with our previous publications would not misconstrue these statements to mean "complete elimination of *all* Langerhans cells." For the record, I agree with Dr. Cruz that both UVB-treated and tape-stripped epidermis contain LC—although I do not agree that the remaining cells can be expected to have normal functional properties.

Dr. Cruz has misinterpreted or confused published evidence concerning the in vivo immunogenic properties of purified populations of epidermal LC. We have demonstrated that as few as 10 allogeneic LC can sensitize some recipient mice [1], but the assay we used was splenic CTL generation, not delayed or contact hypersensitivity. To my knowledge, the lowest number of "pure" hapten-derivatized LC that has been shown to be capable of inducing contact hypersensitivity in vivo is 6,000 [2] or 5,000[3]. Nevertheless, the point Dr. Cruz makes—that residual LC could contribute to the sensitization generated after DNFB-painting of tape-stripped mice—is a valid one to consider, and perhaps I was remiss in omitting it from the *Discussion* in the article in question. However, each of us must be given the "space" to make our own judgments about which experimental evidence is to be considered "signal" and which is to be considered "noise." To my mind, the few LC that remain after tape stripping are unlikely to be immunologically important in induction of contact hypersensitivity, because they appear to be of little consequence in allografts [4]. I would point out that this is the antigen system that correlates with our finding that 10 allogeneic LC can induce CTL. Because tape-stripped grafts are very poor