Diminished Antigen-Presenting Function by Freshly Isolated Epidermal Langerhans Cells: Possible Explanations Based on Methodology

To the Editor:

We were surprised by the recent report of Tiegs et al [1], which demonstrated the acquisition of an antigen-presenting function by Langerhans cells (LC) during several days in culture. Our interest was stimulated by these investigators' use of methods derived from our own work [2], by results that on the surface contradict the results in our own recently published studies [3], and by alternative explanations that may be considered.

First, several investigative groups have demonstrated previously that freshly isolated mouse LC are capable of processing and presenting intact protein antigens (Ag) to Ag-specific T cells [3–7]. By contrast, a loss of antigen-processing capacity has been reported in some, but not all, studies that employed short-term (2–4 d) cultured LC. Some investigators reported cultured LC to be able to process and present pigeon cytochrome c (PCC) or ovalbumin (OVA) [4,7,8], whereas others have shown similarly prepared cultured LC to be incapable of processing OVA or myoglobin [5,6].

The paper by Tiegs et al [1] has added a new twist to this question, because they have demonstrated that freshly isolated LC from C3H/HeN mice are incapable of presenting three different proteins (PCC, fowl gamma-globulin, and keyhole limpet hemocyanin [KLH]) to H-2^k-restricted, Ag-specific Th1 and Th2 clones. After 2–4 d in culture (in the continuous presence of Ag), LC acquired the capacity to activate these T cells. In our own studies, we have demonstrated FACS-purified freshly isolated LC from BALB/c mice to be fully capable of processing and presenting KLH to Ia^d-restricted KLH-specific Th1 or Th2 [3]. In fact, a short pulse of freshly isolated LC with KLH (50–100 μg/ml, 1 h, 37°C, 5% CO_2) was sufficient to induce optimal Th1 or Th2 proliferation.

Could differences in methods explain these discrepancies? Tiegs et al obtained EC suspensions by incubating skin for 45 min in a 0.5% trypsin solution (presumably at 37°C), which is a "modification" of the overnight incubation at 4°C in 0.3% of buffered trypsin described initially by us [2]. This modification has specific relevance to their results because we have observed trypsinization for 45 min at 37°C to reversibly abrogate the capacity of BALB/c LC to present KLH to both Th1 and Th2 (Simon et al, unpublished results). In this respect, it would be important to find out whether freshly isolated LC, obtained by overnight trypsinization, also fail to present KLH to Th1 cells.

Could the strains of mice employed explain their results? Important work by Aiba and Katz has demonstrated strain-dependent differences in antigen-presenting capability: cultured LC from different Ia^k-restricted mice processed and presented intact PCC, OVA, or hen egg lysozyme to Ag-specific T cells; by contrast cultured LC from Ia^d mice were incapable of this function [9]. In light of these findings, it would be important to determine whether freshly isolated LC from Ia^d-restricted mouse strains in Tiegs' model also fail to process and present protein Ag.

In summary, we emphasize the possibility that the temporary processing defect of freshly isolated LC reported by Tiegs et al may be due to effects of a harsher trypsinization protocol and/or reflect a unique property of Ia^k-restricted mouse strains.

Jan C. Simon, Paul R. Bergstresser, and Ponciano D. Cruz, Jr.
Department of Dermatology
University of Texas Southwestern Medical Center
Dallas, Texas

REFERENCES

1. Tiegs SL, Evavold BD, Yokoyama A, Stec S, Quintas J, Rowley D: Delayed antigen presentation by epidermal Langerhans cells to cloned Th1 and Th2 cells. J Invest Dermatol 95:446–449, 1990
2. Sullivan S, Bergstresser PR, Tigelaar RE, Streilein JW: FACS purification of bone-marrow derived epidermal populations in mice: Langerhans cells and Thy-1+ dendritic cells. J Invest Dermatol 84:491–495, 1985
3. Simon JC, Cruz PD, Bergstresser PR, Tigelaar RE: Low-dose UVB-irradiated Langerhans cells preferentially activate CD4+ cells of the Th1 subset. J Immunol 145:2087–2091, 1990
4. Shimada S, Caughman SW, Sharrow SO, Stephany D, Katz SI: Enhanced antigen-presenting capacity of cultured Langerhans cells is associated with markedly increased expression of Ia antigen. J Immunol 139:2551–2555, 1987
5. Streilein JW, Grammer SF: In vitro evidence that Langerhans cells can adopt two functionally distinct forms capable of antigen presentation to T lymphocytes. J Immunol 143:3925–3933, 1989
6. Romani N, Koide S, Crowley M, Witmer-Pack M, Livingstone A, Fathman CG, Inaba K, Steinmann RM: Presentation of exogenous protein by dendritic cells to T cell clones. J Exp Med 169:1169–1178, 1989
7. Picut CA, Lee CS, Dougherty BA, Anderson KL, Lewis RM: Immunostimulatory capabilities of highly enriched Langerhans Cells in vitro. J Invest Dermatol 90:201–206, 1988
8. Hauser C, Katz SI: Activation and expansion of hapten- and protein-specific T helper cells from nonsensitized mice. Proc Natl Acad Sci 85:5625–5628, 1988
9. Aiba S, Katz SI: Cultured epidermal Langerhans cells can process and present intact protein antigens (abstr). J Invest Dermatol 94:502, 1990

REPLY

In addition to the interesting points raised by Simon et al I would like to comment on differences in methodology and address how these could potentially account for discrepancies in our results [1,2]. I have obtained EC suspensions by two methods: the first, which employs an overnight incubation in 0.3% Trypsin/GNK buffer, strictly adheres to the methodology used by Simon/Tigelaar et al and was learned by Tiegs in the laboratory of Nixon-Fulton/Tigelaar [3] and the second "modified" method, which employs a 45-min incubation in 0.5% trypsin. I have used LC obtained by both methods and have obtained very reproducible results. The second "modified" method has also been widely and successfully employed by several other laboratories experienced in epidermal cell work to isolate both LC and DETC [4]. As a preliminary to my investigations we also tested the effects of my modified trypsinization protocol on disaggregated suspensions of splenocytes and found that the trypsin incubation did not abrogate the antigen-presentation function. Furthermore, trypsinization of LC that had first been pulsed with antigen in culture did not alter their ability to function as APC. Therefore I disagree with the suggestion by Simon et al that the "modified" trypsinization procedure is responsible for the differences in our findings.