Third International Workshop on Langerhans Cells: Discussion Overview

Paul R. Bergstresser, Ponciano D. Cruz, Jr., Jerry Y. Niederkorn, and Akira Takashima

Departments of Dermatology and Ophthalmology and the Immunology Program, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.

To promote the exchange of information about ideas and techniques, the Third International Workshop on Langerhans Cells was organized to include relatively long periods of time for discussion. Although we had originally intended to publish an edited version of that discussion, it became obvious that much of the material was covered by each manuscript. As a compromise, the pages that follow provide a brief review of the discussion, focusing on important issues, problems, and questions that emerged. Our goal is to provide readers interested in the biological and immunological aspects of Langerhans cells (LC) with an overview that allows them to place the entire Workshop into perspective. We have organized this review around six noteworthy issues concerning Langerhans cells, including 1) their "true" identity, 2) migratory properties, 3) cytokine profiles, 4) Fc receptors, 5) interactions with biologic antigens, and 6) mechanisms by which UVB radiation induces immunosuppression.

The workshop was set into lively motion by Dr. Rudolf L. Baer, a distinguished colleague, who has served as scientific investigator as well as a facilitator of the work of others during the two decade-long explosion of knowledge about LC.

WHAT IS THE "TRUE" IDENTITY OF LANGERHANS CELLS?

As new data about LC have accumulated, investigators have become less convinced that their defining features are either uniform or stable. A decade ago, LC were characterized as resident epidermal cells that exhibit a dendritic morphology in situ, express cell-surface MHC class II antigens, CD1 and ATPase, and contain a characteristic microstructure, the Birbeck granule. Although these features did describe the overwhelming majority of LC in normal epidermis, they and other more recently reported features fall short when employed to address contemporary questions, such as ontogeny, migratory properties, and responses to exogenous stimuli. In fact, as will be demonstrated in the following, it is now certain that LC, as a class of dendritic cells (DC), are not uniform in either phenotype or function. In addition, mechanisms for the expression of these different phenotypes is not certain, giving rise to two non-exclusive possibilities, that DC divide into several permanent and distinct lineages and/or that they are a single population, the members of which acquire unique states of activation, depending on their location and/or their recent experience. This interaction between phenotype and function figured prominently in the course of the workshop.

The issues of subsets and lineage do not begin with LC, rather they begin with the special relationship between LC and DC, including both their similarities and their differences. First, several areas of agreement were reached concerning the current understanding of DC. In terms of phenotype and function, it was agreed that DC exhibit 1) a veiled or dendritic morphology, 2) modest phagocytic capacity, and 3) potent capacity to activate naive T cells. Moreover, investigators agreed with a general concept of DC life history; they 1) are derived from precursors in bone marrow, 2) distribute via the blood to selected tissues, 3) home to their respective tissues (for LC this might be immediately or after an unknown period of retention in the dermis), 4) leave that tissue and migrate toward draining lymph nodes following an activation step associated with exposure to an antigen (Ag), 5) mature into more "potent" DC, and 6) stimulate selected but immunologically naive T cells upon entry into lymph nodes.

The next relevant question concerned characterization of DC subsets. In fact, at least five distinct subsets were discussed in the workshop: 1) LC in epithelial tissues, 2) DC in peripheral blood or lymph, 3) interdigitating DC in T-cell – dependent areas of lymphoid tissues, 4) follicular DC in B-cell – dependent areas, and 5) dermal dendrocytes or perivascular DC in dermis.

Peripheral blood DC are identified routinely by their capacity to exhibit by phase-contrast microscopy a "veiled" or "dendritic" morphology. DC have also been identified by their cell-surface phenotype, including the constitutive expression of large amounts of HLA-DP, -DQ, and -DR, the high-affinity IL-2 receptor, and low levels of CD4 and CD40.

Returning to LC as a class, data presented in the workshop clearly illustrated that LC are not stable; rather, they exhibit considerable dynamism, migrating into and out of epidermis, and modifying both their phenotype and critical aspects of their function, depending on their location, state of maturation, and the stimulatory factors provided. One attractive and frequently discussed idea has been that resident LC in epidermis are "immature" and that they "mature" in tissue culture as they acquire characteristic features of classic DC [1,2]. These observations have given rise to the hypothesis that similar maturational steps occur as LC leave the epidermis.

On the other hand, dermal dendrocytes may represent "immature LC." These cells have been characterized by their preferential localization around blood vessels, and by their expression of HLA-

Reprint requests to: Dr. Paul R. Bergstresser, Department of Dermatology, U.T. Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

Abbreviations:
Ag: antigen
APC: antigen-presenting cell
DC: dendritic cell
GM-CSF: granulocyte macrophage/colony-stimulating factor
HIV: human immunodeficiency virus
LC: Langerhans cell
PCR: polymerase chain reaction
RT-PCR: reverse transcriptase followed by PCR
TNFα: tumor necrosis factor-α
UCA: urocanic acid
UVB: ultraviolet B

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DR, CD1a, CD1b, CD1c, and CD11c (Cooper, Furue*). By contrast, LC in epidermis express HLA-DR, CD1a, faint CD1c, FcεRII/CD23, and FcγRII/CD32, but not CD1b or CD40. Because of potential effects of procurement techniques, including trypsinization, the question whether LC express adhesion molecules such as ICAM-1, LFA-3, CD11a, CD11b, CD11c, or CD18 in situ has not been answered, whereas cultured human LC certainly express some of these molecules (Teunissen).

Ultimately, a discussion of LC identity leads to critical questions concerning relationships among the different DC subsets. At which points do the lineages of monocytes, DC, and LC diverge? Do DC subsets represent cells of recently divergent lineage or do they simply represent the same cell with phenotypes that reflect differences in location? Are dermal dendrocytes precursors of epidermal LC, and, if so, do they change their phenotype before or after gaining entry into the epidermis? Is the epidermal microenvironment (e.g., cytokines and adhesion molecules) responsible for this change? With regard to LC precursors, it was reported that hapten painting causes the appearance in dermis and epidermis of "immature LC" that were distinct from dermal dendrocytes and from epidermal LC (Kolde).

WHAT SIGNALS FACILITATE THE MIGRATION OF LANGERHANS CELLS?

A large body of evidence now documents the ability of LC to move. This evidence began to accumulate more than a decade ago with the observation that LC are derived from bone marrow precursors [3], implying that they must subsequently move to skin (presumably to dermis via the blood stream, and then to epidermis by chemotaxis). More recently, studies with corneas have demonstrated LC to exhibit rapid lateral chemotactic responses to IL-1 (Niederkorn). Finally, hapten painting can induce LC to depart from epidermis and to enter draining lymphatics, eventually reaching regional lymph nodes within a short period of time [4]. Studies presented in the workshop addressed several aspects of these processes. First, LC precursors from peripheral blood were observed to migrate into human skin grafts that had been reconstituted in vivo in immunodeficient mice (Rowden, Démarchez). Secondly, it was reported that LC procured from trypsin-disaggregated mouse epidermis retained their capacity to migrate back into epidermis (Cruz, Saitoh). Finally, after LC gain entry to the epidermis, residence may be facilitated by their marked adhesiveness to keratinocytes (Takashima).

Two laboratories reported elegant in vivo methods for monitoring LC movement by cannulating lymph vessels that drain sites of skin painting. These data indicate that painting with allergic or irritant contact sensitizers, or with chemical carcinogens, produces rapid migration of LC into afferent lymphatics (Brand, Dandie). These observations led to a lively discussion concerning the ultimate fate of these cells, once they gain entry into a regional lymph node. How long do they reside there? Do they survive and return to the epidermis? From the discussion, the consensus was that the majority of emigrating LC are retained in the first draining lymph node that is reached. DC (and perhaps LC) may serve as targets of natural killer cells (or of cytotoxic T cells), suggesting that LC are destroyed after they have stimulated T cells. On the other hand, as many as 1% of cells in the thoracic duct are DC, thereby suggesting the opposing view that a significant number of LC survive and recirculate.

Important questions were raised concerning the signals that ordinarily produce LC movement. IL-1 is clearly one candidate, particularly with respect to movement into the epidermis (Niederkorn). With respect to movement out of the epidermis, a role for tumor necrosis factor-α (TNFα) was proposed; intradermal injection of TNFα causes a significant increase in the number of DC found within draining lymph nodes (Kimber). On the other hand, this conflicted with observations suggesting that TNFα may immobilize LC within the epidermis [5].

Issues of LC adhesion within the extracellular matrix also figured prominently in the discussion. With respect to the specific adhesion molecules that might promote migration, it was reported that LC have the capacity to bind to extracellular matrix proteins via β1 integrins (Staquet). Much remains to be clarified, however, including the question of whether this adhesion through receptors promotes or retards cell movement.

WHAT ROLES ARE PLAYED BY CYTOKINES IN LC FUNCTION?

Cytokines are now recognized to play important roles in the initiation, amplification, and termination of immunologic reactions. The seminal observation that keratinocytes elaborate a cytokine indistinguishable from IL-1 [6] was only the beginning of presently continuing discoveries of a large catalog of proteins, both cytokines and growth factors, produced by these cells. Some of these factors regulate LC functions, including their migration, expression of surface molecules, and survival. Aside from the early observation that IL-1 activity detected in culture supernatants was secreted by both keratinocytes and LC [7], little has been reported concerning the profile of cytokines produced by LC alone.

New information is now on the horizon. Three laboratories reported mouse LC to express mRNA for IL-1β, MIP-1α, and IL-6, as detected by reverse transcriptase followed by polymerase chain reaction (RT-PCR) (Enk, Schreiber, Matsue). These important reports led to questions concerning the care that must be taken when employing a sensitive detection technique such as PCR, and when interpreting results that are obtained. Minor contaminating populations may be inadvertent sources of detected mRNA. In overcoming this problem, it was recognized that the use of LC lines and clones derived from epidermis, although ideal in terms of cell purity, would have the disadvantage of not reflecting in situ levels of mRNA. Ultimately, rapidly procured and highly enriched populations of LC will be required.

Unfortunately, even rapid techniques of LC isolation may have substantial effects on mRNA levels; within 15 min as much as a hundredfold increase in message for IL-6 has been observed in epidermis as compared with "baseline" levels. Compounding this problem, it was also argued that mRNA expression may be "upregulated" by artifactual stimuli. The first example concerned the highly relevant question of whether experimental animals are pathogen-free. The second example concerned whether the reported "constitutive" expression of IL-1β, for instance, has been induced by trace amounts of lipopolysaccharide that may contaminate buffer solutions or culture media. Thus, as assays for mRNA and for proteins become more sensitive, these questions will recur repeatedly. The obvious conclusion from the workshop was that at present it is difficult to ascertain that "resting" LC in situ express the same cytokine mRNA profiles as revealed by in vitro analyses.

Taken together, these studies led investigators to recognize that the paracrine cytokine network within epidermis should be studied along two directions: keratinocyte-derived cytokines that regulate the activities of LC and LC-derived cytokines that regulate keratinocytes. Finally, the ultimate issue concerns how exogenous stimuli may regulate cytokine production by LC. For example, it was reported that hapten painting modulates cytokine mRNA profiles in epidermis, of which LC are responsible for upregulated IL-1β (Enk).

WHAT IS THE FUNCTION OF FC RECEPTORS EXPRESSED ON LC?

Important new knowledge has been forthcoming concerning the presence and function of Fc receptors on LC. This set of issues dates back to the observation that human LC are capable of binding Fc fragment of IgG [8]. In this respect, three distinct receptor molecules have now been identified in human leukocytes, FcγRI/CD64, FcγRII/CD32, and FcγRIII/CD16. It has been previously shown that FcγRII is responsible for IgG binding to LC [9]. A novel obser-

*References to abstracts and papers from the workshop are made by citing the first author.

vation reported in the workshop was that LC express FcγII not only on the cell surface, but also secrete this molecule into culture media (de la Salle). These investigators have cloned two forms of cDNA from human LC: one encoding FcγII that contains the transmembrane domain (membrane-associated form), the other external to this domain (soluble form). Moreover, mRNA expression of these two forms of FcγII appears to be regulated by TNFα. Many questions were raised concerning the physiologic significance of these findings. Does a membrane form of FcγII mediate binding and internalization of antigens via IgG? Does a soluble form serve as an inhibitor in this function? How are FcγII expression and secretion regulated in LC in situ?

Human LC also possess IgE-binding capacity, and early studies demonstrated LC in atopic dermatitis patients to bind IgE [10]. On the other hand, the specificity of this phenomenon for atopic dermatitis cannot be assured, as it has been shown that LC in other diseases with elevated IgE also exhibit this function [11]. Two IgE-binding structures were previously found on human LC; FcεRII/CD23 (low-affinity receptor) and εBP (so-called IgE-binding protein) [12]. In the workshop, two laboratories demonstrated that human LC also express the high-affinity IgE receptor, FcεRI (or an FcεRI analogue with different molecular configuration), by using RT-PCR and by specific monoclonal antibodies (Bieber, Rieger).

These findings stimulated discussion about the functional significance of IgE receptors on LC, and new evidence is beginning to emerge. In fact, IgE-mediated Ag presentation has been reported in LC from atopic dermatitis patients [13]. It was also suggested that cell-surface IgE may serve as a receptor for certain Ag, thereby mediating high-affinity and selective binding of Ag, and/or effective internalization of these molecules. Another possibility is that crosslinked IgE may stimulate the secretion of cytokines by LC. Additional discussion concerned how the expression of IgE receptors is regulated. First, it would appear that not all LC, even in atopic dermatitis, express FcεRI, and LC during culture appear to lose FcεRI. The finding that FcεRII expression by LC can be upregulated by IL-4 and IFNγ [12] suggests that cytokines secreted by epidermal cells may modulate IgE receptor turnover in LC.

BY WHAT MECHANISMS DO LC INTERACT WITH BIOLOGIC ANTIGENS?

One property of LC retained central focus throughout the workshop: their capacity to present Ag to T cells with extraordinary efficiency. These Ag include chemically reactive haptens, proteins, and allogeneic cellular determinants. As might be expected, the discussion centered primarily on infectious agents, haptens and tumors. Considerable discussion was prompted by the report that mouse LC are capable of processing and presenting tumor antigens to $CD4^+$ T cells and that this activity is highly regulated by cytokines [e.g., IL-1α, granulocyte macrophage/colony-stimulating factor (GM-CSF), and TNFα] that are secreted by keratinocytes (Grabbe). This gave rise to questions about the role LC play in immune surveillance against tumors. Can one induce protective immunity against tumors by the adoptive transfer of such $CD4^+$ T cells or through immunization with tumor-pulsed LC? It was remarked that immunization of naive animals with tumor-pulsed LC causes a delay of growth of inoculated tumor cells [14]; this protocol, however, was less effective in animals that have already carried tumors. It was noted that spleen DC pulsed with tumor cells are also capable of producing protective immunity (Knight).

Clinical findings that LC density is increased or decreased in skin lesions of American cutaneous leishmaniasis, depending upon the immunologic responses to the parasites (Càceres-Dittmar), raised several important questions. Does this mean that LC are targets of parasite infection or that they are responsible for presenting parasite Ag to T cells? In fact, infection and replication of *Leishmania major* within LC has been reported [15]. Which T-cell subset (Th1 or Th2) is primarily activated? It was mentioned that PCR analyses of cytokine mRNA present in skin lesions failed to clarify this issue, revealing cytokine profiles of Th0 type instead. Does ultraviolet B (UVB) radiation modulate parasite infection and/or subsequent immune reaction? Obviously, this question is difficult to address clinically, because sun-exposed areas of the body are also the sites frequently bitten by the leishmania vector, the sandfly. On the other hand, this question can be addressed in murine models of Leishmaniasis (Sànchez).

The role of LC in human immunodeficiency virus (HIV) infections was given much attention in the workshop. Beginning with the seminal observation of Tschachler [16], that HIV are found in LC, the question that reverberated was whether LC are a relevant target for HIV, taking into account the fact that perhaps only a small proportion of LC in skin of $HIV\text{-}1^+$ patients are infected with the virus. In the course of the discussion, three major issues were considered. 1) Can LC be infected with HIV? 2) If so, does CD4 molecule on LC serve as a receptor for HIV entry? 3) Do HIV replicate in LC to be transmitted to other leukocytes? Studies using in vitro infection systems did demonstrate that human LC, but not other epidermal cells, can be infected by HIV-1 (Berger, Dusserre). Moreover, viral replication and transmission to lymphocytes were observed in these systems, suggesting that LC serve not only as targets for HIV-1, but also as viral reservoirs and vectors for transmission, as has been demonstrated for peripheral blood DC (Langhoff).

BY WHAT MECHANISM(S) DOES UVB RADIATION INDUCE IMMUNOSUPPRESSION?

UVB radiation is known to downregulate T cell–mediated immunity in skin. Hapten application through UVB-irradiated skin results in the failure to immunize, and animals so treated can no longer be sensitized with the same hapten at a later date, even through normal skin [17]. Postulated mechanisms for this immunosuppressive effect have received considerable attention. The simplest explanation is that UVB radiation kills LC, and sensitization is not achieved because they are no longer available in epidermis. In fact, it was reported that in vitro exposure of mouse LC to fluences of UVB that ordinarily inhibit APC activity cause LC death 48–72 h after UVB irradiation (Tang). This observation prompted the following questions. How can the tolerance be induced without antigen-presenting cells (APC)? Do LC retain some aspects of antigen-presenting capacity in the interval before cell death? If so, does UVB cause a deficiency in cytokine production or a deficiency in co-stimulatory molecules on LC, that in turn leads to clonal anergy of T cells (Jenkins)? Does UVB cause influx of alternative APC that subsequently are responsible for immunosuppression?

A second series of questions concerned the chromophores that are responsible for UVB-induced immunosuppression. Is DNA the sole target? What are the mediators responsible for modulation of APC function of LC? In fact, urocanic acid (UCA) in stratum corneum has been postulated to serve as a chromophore through photoisomerization of naturally occurring trans-UCA into cis-UCA. Cis-UCA, in turn, has been reported to induce suppression in a variety of experimental circumstances, including through its topical application [18]. Topical application of cis-UCA was reported at the workshop to produce morphologic changes and emigration of LC (Kurimoto, Norval).

Finally, TNFα was discussed at length as a candidate molecule responsible for UVB-induced immune dysfunction. UVB radiation does induce increased secretion of TNFα by keratinocytes [19] and TNFα does exert immunosuppressive effects in skin [20]. One unifying proposal was that cis-UCA might serve as the chromophore to induce TNFα elaboration, which, in turn, would be responsible for the actual immunosuppression. This proposal was supported by the observation that anti-TNFα antibodies reversed the immunosuppressive effects induced either by UVB radiation or by cis-UCA (Kurimoto). This raised the unanswered question whether cis-UCA has the capacity to upregulate TNFα secretion by keratinocytes. Ultimately, it is likely that UVB produces multiple effects in skin, and it is too early to be confident that a single mechanism, albeit

through DNA, UCA, or some as yet unidentified substance, predominates.

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