Brief Definitive Report

EVIDENCE OF HLA-DR ANTIGEN BIOSYNTHESIS BY HUMAN KERATINOCYTES IN DISEASE

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Whereas, within normal murine and human epidermis, the expression of class II alloantigens is restricted to bone marrow–derived Langerhans cells (LC) (1), several investigators have recently reported on the detection of class II alloantigens on keratinocytes (KC) in certain disease states, e.g. cutaneous T cell lymphoma (CTCL) (2), lichen planus (LP) (2), and cutaneous graft-vs.-host disease (GVHD) (3). The emergence of HLA-DR antigens on primarily DR⁻ KC raises the question about their cellular origin. The possibility that they might be synthesized, rather than passively acquired, gains support from animal studies showing that induction of GVHD in irradiated F_1 hybrid rodents by the injection of parental lymphocytes results in the appearance of KC Ia antigens of host phenotype (4, 5). Due to careful HLA-matching, in human bone marrow transplantation haplotypic differences or identities between KC-bound and donor cell–derived Ia antigens cannot be evaluated. Our strategy to clarify the cellular origin of Ia antigens on human KC was therefore based on different considerations. HLA-DR antigens represent the human analogue to murine I-E antigens (6), whereas HLA-DS (7) or DC1 antigens (8) correspond to murine I-A molecules. HLA-DR oligomers are formed by the noncovalent association of three polypeptides, i.e., α, β, and γ chains (9). As opposed to α and β chains the γ chain appears to be invariant, at least by charge and mass, regardless of the DR phenotype (10, 11). Cell surface HLA-DR molecules consist only of α/β-dimers, whereas γ chains appear to be part of HLA-DR oligomers exclusively during cytoplasmic processing (12) and are apparently needed for the intracytoplasmic transport of HLA-DR (9). We thus felt that demonstration of the HLA-DR γ chain within a cell carrying HLA-DR α and β surface moieties would provide strong evidence for active HLA-DR biosynthesis by this given cell.

The monoclonal antibody VIC-Y1 (13) defines an antigenic determinant carried exclusively by cytoplasmic HLA-DR γ chains, both free and in association with HLA-DR α and β oligomers (14). We now describe the occurrence of HLA-DR antigens on KC in disease states and demonstrate that KC carrying surface-bound HLA-DR antigens exhibit cytoplasmic staining when exposed to VIC-Y1 in an indirect immunofluorescent (IF) technique. We therefore propose that HLA-DR antigens on KC of diseased skin are actively synthesized by these cells.

TABLE I

Monoclonal Antibodies

Monoclonal antibody	IgG Class	Antigenic determinant	Working dilutions
Anti-HLA-DR (15) (Becton/ Dickinson (BD)) unconju- gated, FITC- or TRITC- labeled	IgG$_{2a}$	Nonpolymorphic determi- nants on the DR α/β com- plex	1:40
VIC-Y1 (13, 14)	IgG$_1$	Cytoplasmic DR γ chain	1:10
Anti-Leu-10 (16) (BD)	IgG$_1$	α/β chains of non–HLA-DR molecules co-migrating with DC-1 molecules	1:5
OKT6 (17) (Ortho Diagnos- tic Systems Inc., Raritan, NJ)	IgG$_1$	T6 antigen (cortical thymo- cytes, epidermal LC)	1:10

Material and Methods

Biopsy Specimens. Excisional biopsies were obtained from lesional skin of patients with LP (13 cases), CTCL (6 cases), and chronic cutaneous GVHD (5 cases), which had developed after allogeneic bone marrow transplantation. Skin specimens from patients undergoing corrective plastic surgery served as control. The tissues were embedded in OCT compound (Tissue Tek, Ames Co., Elkhart, IN), snap-frozen in isopentane cooled in liquid nitrogen and stored at $-70°C$.

Monoclonal Antibodies. (See Table I.) Binding of unconjugated monoclonal antibodies was visualized by affinity-purified fluorescein isothiocyanate (FITC) goat F(ab')$_2$ anti-mouse IgG (Tago Inc., Burlingame, CA; working dilution 1:10 in PBS).

Immunofluorescence. 4-μm cryostat skin sections were air-dried, acetone-fixed, and rinsed in phosphate-buffered saline (PBS), pH 7.2. For direct IF studies, sections were incubated with either FITC- or tetra-methyl-rhodamine isothiocyanate (TRITC)-labeled anti–HLA-DR for 30 min. In indirect IF assays, sections were first exposed to appropriate dilutions of anti–HLA-DR, anti–Leu-10, VIC-Y1, OKT6 monoclonal antibodies, or nonimmune ascites as a control, and antibody binding was visualized by a 30-min incubation with FITC goat F(ab')$_2$ anti-mouse IgG. When double-labeling was attempted, the sections were incubated with 20% normal mouse serum, washed, and then exposed to appropriate dilutions of TRITC-anti–HLA-DR (30 min). After final PBS washes, sections were air-dried, mounted with FA mounting fluid (Difco Labs, Detroit, MI), and examined under a Leitz Ortholux fluorescent microscope.

Results

Normal Human Skin. Despite differences in binding patterns within the dermis, the epidermal reactivity of all monoclonal reagents (anti–HLA-DR, anti–Leu-10, OKT6, VIC-Y1 [Fig. 1]) is restricted to dendritic cells whose shape and distribution identify them as LC and indeterminate cells, respectively.

Diseased Skin. Compared to the frequency of OKT6$^+$/HLA-DR$^+$/HLA-DS$^+$/VIC-Y1$^+$ LC and indeterminate cells within normal epidermis, their number was slightly increased in LP and CTCL specimens but appeared decreased in GVHD epidermis. The most conspicuous feature in sections of 12/13 LP, 5/6 CTCL, and 5/5 GVHD patients was the emergence of HLA-DR antigens on KC of virtually all layers of the epidermis. The uniform membrane staining of KC exposed to anti–HLA-DR was contrasted by their lack of reactivity with anti–Leu-10 (Fig. 2, *a* and *b*). In LP and CTCL biopsies, expression of surface HLA-DR was most pronounced on KC overlying dense mononuclear cell infiltrates, whereas in GVHD samples virtually all KC displayed uniform surface staining.

When serial sections of the same biopsies were incubated with VIC-Y1, we
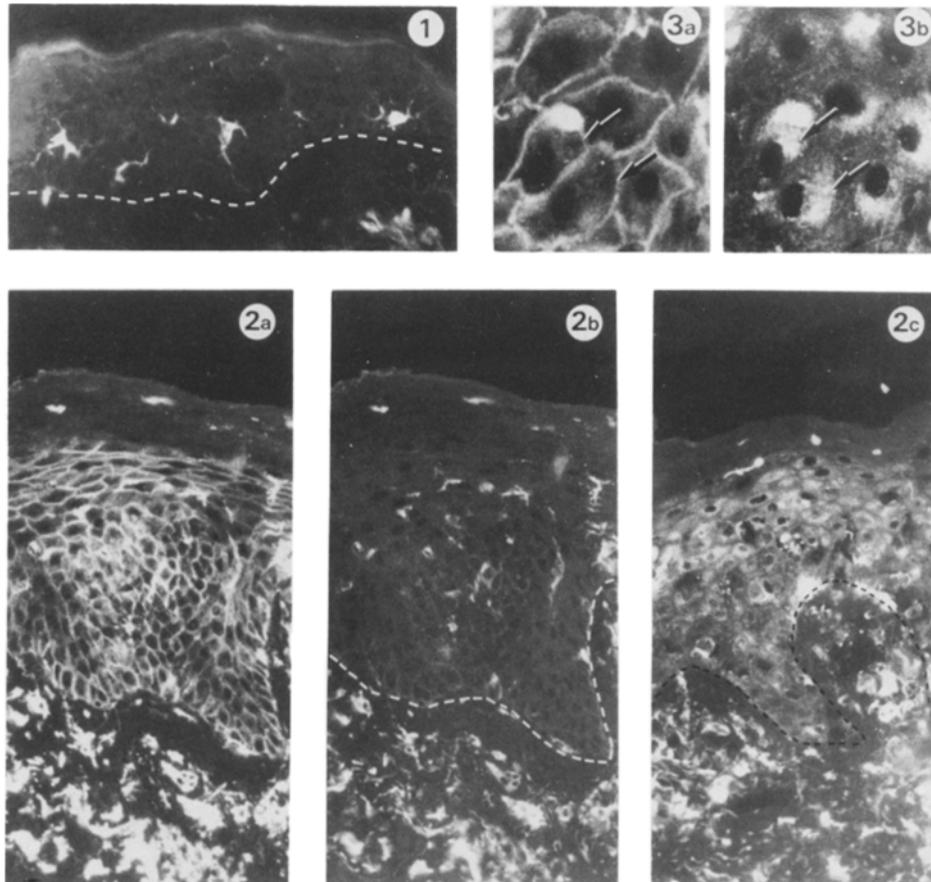
FIGURE 1.   Reactivity pattern of VIC-Y1 with normal human skin: cytoplasmic staining of dendritic suprabasal LC within the epidermis. FIGURE 2. (a–c) CTCL, stage IA: staining of cryostat sections with (a) TRITC-labeled anti-HLA-DR: distinct surface staining of KC in a netlike pattern. Beneath the dermo-epidermal junction, the infiltrate contains abundant anti–HLA-DR reactive cells. (b) Staining of an adjacent section with anti–Leu10: KC remain negative, while staining of epidermal LC and dermal infiltrating cells can be observed (the dashed line indicates the dermal-epidermal junction). (c) VIC-Y1 in a serial section of the same biopsy specimen: cytoplasmic, fluorescence within the majority of KC but no surface staining. FIGURE 3. (a, b) Surface-bound HLA-DR α/β complexes (a-arrow; anti-HLA-DR, TRITC) and intracytoplasmic HLA-DR γ chain (b-arrow; VIC-Y1, FITC) by double labeling.

found that, within corresponding areas of the specimens, KC displayed homogenous cytoplasmic fluorescence sparing the nuclear region and the intercellular spaces (Fig. 2c). Double staining procedures revealed that anti–HLA-DR and VIC-Y1 reactive antigenic determinants are located on the surface and within the cytoplasm, respectively, of the very same KC (Fig. 3, a and b). Controls with nonimmune ascites as first-step reagent were completely devoid of staining.

Discussion

The intracytoplasmic demonstration of HLA-DR γ chains within the KC bearing surface-bound HLA-DR α and β chains provides strong evidence for the active biosynthesis of HLA-DR antigens by these cells for the following reasons.

During HLA-DR biosynthesis, oligomeric assembly of DR α, β, and γ chains occurs shortly after their synthesis in the endoplasmic reticulum as pulse-chase experiments have revealed that by immune precipitation with either anti–HLA-DR α/β reagent (9) or VIC-Y1 (14), all three polypeptide chains are co-precipitated. DR γ chains present in the oligomeric complex with DR α and β moieties undergo intracellular transport and, subsequently, are detached from the oligomeric complex that finally appears on the cell surface (9, 14). The ultimate fate of the DR γ chain is unknown. Although Koch et al. (18) recently reported on the detection of the murine Ia invariant chain on lymphocyte surfaces, most authors agree that its presence is restricted to the cytoplasm (9, 12). Our numerous attempts to detect surface reactivity of VIC-Y1 by immunoelectron microscopy and flow cytofluorometry consistently yielded negative results (14). We therefore propose that the human DR γ chain or at least its VIC-Y1 reactive portion does not appear on the cell surface and that VIC-Y1 is a selective marker for cytoplasmic HLA-DR. The virtually complete correlation of VIC-Y1 cytoplasmic and anti–HLA-DR surface reactivity again points to the important role of γ chains in HLA-DR biosynthesis. Since, however, the murine invariant chain gene is not included in the MHC-complex (19), one may argue by analogy that, in the disease states described, yet unknown stimuli may induce KC to synthesize DR γ chains without concomitant induction of DR α/β synthesis. Such an event has recently been reported with human and murine plasmacytoma cells (20). Consequently, one would have to concede that KC surface HLA-DR α/β chains were passively absorbed from surrounding DR-positive cells, which in the course of the respective disease process, either had become severely damaged, or alternatively, had been induced to synthesize excess amount of DR α/β chains. Although the exchange of Ia determinants between cells has been reported (21), we consider this possibility rather unlikely, mainly because of our finding that anti–HLA-DR reactive and VIC-Y1 reactive KC were essentially identical.

When we thus assume that KC-bound surface HLA-DR in diseased skin is actively synthesized by these cells, the stimuli leading to this event have yet to be clarified. There is ample evidence that Ia antigen expression on murine macrophages can be modulated by soluble factors released by T cells after antigen or mitogen activation. In this regard, particular attention has focused on γ-interferon which—in contrast to other cytokines tested—can induce Ia expression on primarily Ia$^-$ macrophages (22) and umbilical vein endothelial cells (23). It is thus conceivable that, in the disease states listed, activated T cells within the dermal infiltrate produce quantities of γ-interferon sufficient to induce HLA-DR biosynthesis by KC.

The appearance of HLA-DR, but not HLA-DS antigens on primarily DR$^-$ cells may shed some light on the role of these alloantigens in the induction of T cell–dependent immune responses. Normal DR$^-$ KC are not capable of inducing proliferative T cell responses (24). The question of whether or not the acquisition of DR moieties by KC widens the repertoire of their functional capacities is currently under investigation.

Summary

As opposed to normal human skin where HLA-DR expression is restricted to the Langerhans cell (LC) population, HLA-DR, but not HLA-DS antigens can

be readily detected on keratinocytes (KC) in certain disease states, i.e., cutaneous T cell lymphoma (CTCL), graft-vs-host disease (GVHD), and lichen planus (LP). To clarify the cellular origin of KC-bound HLA-DR antigens, we used a monoclonal antibody directed against determinants solely expressed on the cytoplasmic HLA-DR γ chain (VIC-Y1) and observed that, by immunofluorescence, KC displaying HLA-DR α/β complexes on their surface uniformly displayed cytoplasmic VIC-Y1 reactivity. In view of the crucial role of the γ chain for HLA-DR biosynthesis, we conclude that HLA-DR antigens on KC are actively synthesized by these cells.

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