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Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens

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SUMMARY

With indirect immunofluorescence techniques we demonstrated that recombinant gamma-interferon induced the expression of the class II antigens HLA-DR and HLA-DQ as well as intercellular adhesion molecule-1 (ICAM-1) on normal, cultured human keratinocytes grown in low-calcium, serum-free medium. Each antigen displayed a distinctive cellular staining pattern. HLA-DR was strongly localized to perinuclear zones with intense cell surface expression; HLA-DQ displayed a perinuclear accentuation, but with minimal cell surface staining, and ICAM-1 was strongly expressed in a diffuse cytoplasmic pattern with intense cell surface expression. Keratinocytes grown in medium supplemented with 10% fetal calf serum underwent differentiation, with a diminished expression of all three antigens as compared to those grown in low-calcium, serum-free medium.

These results confirm that gamma interferon can differentially regulate HLA-DR and HLA-DQ expression; that there are probably different biochemical metabolic pathways by which these three molecules are expressed on keratinocytes, and that the expression is also a function of the degree of keratinocyte differentiation. The strong cell surface expression of ICAM-1 is suggested to be of major importance as the recognition molecule, by which T cells bind to gamma interferon exposed keratinocytes, and suggests an integral role for this molecule in epidermal lymphocyte trafficking.

The human class II major histocompatibility complex (MHC) antigens are coded for by three distinct loci on chromosome six: HLA-DR, HLA-DQ and HLA-DP.[1] Unlike class I MHC

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antigens, which are ubiquitously expressed, class II antigens have a limited cellular distribution on human nucleated cells.[2,3] HLA-DR expression on macrophages is necessary for antigen to be presented to T lymphocytes,[4] and for induction of cytolytic T lymphocyte activity,[5] and is involved in the autologous mixed lymphocyte reaction (AMLR).[6] Much less is known about the functional significance of HLA-DQ. However, it has similar effects to HLA-DR and Gonwa *et al.*[7] have shown it to be required for antigen presentation in the AMLR. In normal skin, both HLA-DR and HLA-DQ are found on the same cells, namely vascular endothelium,[8] Langerhans cells[9] and the acrosyringial epithelium.[10] Under normal circumstances neither antigen is expressed by keratinocytes (KC).[11] In diseased skin, particularly the inflammatory dermatoses, HLA-DR is commonly expressed by KC[12] and has been related to the presence of intra-epidermal lymphocytes.[13] HLA-DQ expression by KC is infrequent although it has been observed in cutaneous T cell lymphoma, lichen planus,[14] *Borrelia*-induced skin disease,[15] and allergic and irritant contact dermatitis.[16]

Gamma interferon IFN-γ, induces HLA-DR expression on normal cultured human KC[17] and direct intradermal injection of IFN-γ will induce the appearance of HLA-DR on the overlying epidermal KC.[18] However, IFN-γ has not been demonstrated to induce HLA-DQ on normal cultured human KC[19] or on KCs in a short-term organ culture system.[11,20]

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein of molecular weight 90–114 kd expressed by both haemopoeitic and non-haemopoeitic cells.[21,22] It is involved in the regulation of lymphocyte–cell adherence mediated by lymphocyte function-associated antigen-1 (LFA-1) which is a ligand for ICAM-1.[23] The adherence of resting and activated autologous and allogeneic T lymphocytes to IFN-γ treated KC is blocked by anti-LFA-1 antibodies[24,25] or anti-ICAM-1 antibody,[26] thus implying a role for ICAM-1 in lymphocyte–KC interactions in the inflammatory dermatoses.

We have investigated the cellular distribution patterns of HLA-DR, HLA-DQ and ICAM-1 in normal human cultured KC in serum-free and serum-supplemented medium and the effect of IFN-γ on the expression of these antigens.

METHODS

Preparation of keratinocyte cultures

Samples of normal human skin were obtained from patients undergoing cosmetic surgery and single cell suspensions prepared according to the method of Liu and Karasek.[27] Small, round, viable cells were seeded onto and grown in either 10 cm diameter Petri dishes (Lux Flow Laboratories, Inc) or Lab-Tek chamber slides (Miles Scientific, Inverhill, IL, U.S.A.) in either a serum-free KC growth medium containing low calcium[28] (KGM, Clonetics, CO., San Diego, CA, U.S.A.) or Dulbecco's modified Eagle's medium (DMEM, Gibco Laboratories, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum (FCS, Gibco). Cells were maintained in a humidified incubator in 5% Co_2/95% air at 37°C and used after passage 3.

Recombinant IFN-γ (specific activity = $1{\cdot}7 \times 10^7$ U/mg; kindly provided by Dr M.Shepard, Genentech Inc., South Francisco, CA, U.S.A.) was used at a final concentration of 1200 U/ml and added to dishes or slides containing sub-confluent KC, after which incubation was continued for 3 days at 37°C.

Staining of keratinocyte for HLA-DR, HLA-DQ and ICAM-1

KC in Lab-Tek chamber slides were either fixed for 15 min in acetone at 4°C to permeabilize the cell membrane prior to staining, or were left unfixed . The KC monolayers were incubated for 25

TABLE 1. Monoclonal antibodies used in the study

Antibody	Specificity	Isotype	Source
TS1.16	HLA-DR	γ1	Dr C.Clayberger, Stanford University U.S.A.
Leu 10	HLA-DQ	γ1	Becton Dickinson, Mountain View, CA, U.S.A.
RR-11	ICAM-1	γ1	Dr T.Springer, Dana Farber Cancer Institute, Boston, MA, U.S.A.
OKT8	CD8	γ1	Ortho Diagnostics, Raritan, NJ, U.S.A.

min on ice with 10μg/ml of the panel of monoclonal antibodies listed in Table 1. OKT8 was used as an isotype irrelevant control antibody. The cells were washed and then stained with 1 μg of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Tago Inc., Burlingame, CA, U.S.A.). In order to investigate the possibility of anti-HLA-DQ antibody binding to HLA-DR antigen, which may have been altered after acetone fixation,[29] KC were stained directly with FITC-conjugated Leu 10 (Becton Dickinson, Mountain View, CA, U.S.A.) for 25 min on ice and compared with KCs pre-incubated with TS 1·16 for 1 h on ice prior to staining with FITC-conjugated Leu 10. The cells were examined using an Olympus BH-2 fluorescence microscope.

Cultured KC were trypsinized using 0·03% trypsin/0·01% EDTA, an aliquot of 10^6 KC was stained as described above, and staining intensity and numbers of cells stained determined using fluorescence activated cell sorter (Epics 541: Coulter Corp), as previously described.[17]

RESULTS

Induction of HLA-DR, HLA-DQ and ICAM-1 antigen expression by IFN-γ

Normal human KC grown in KGM in the absence of IFN-γ did not express HLA-DR, HLA-DQ or ICAM-1. However, the addition of IFN-γ (1200 U/ml) induced distinct patterns of cellular expression for all three antigens. The staining patterns described combine the cell surface staining seen in the absence of fixation or permeabilization, and the intracellular staining seen after acetone treatment.

HLA-DR was localized to the perinuclear zone with intense cell suface expression (Fig. 1), as has been observed previously.[30] HLA-DQ showed perinuclear localization, but with minimal cell surface staining (Fig. 2). ICAM-1 was strongly expressed in a diffuse cytoplasmic pattern with intense cell surface expression (Fig. 3). Using FITC-conjugated Leu 10 (anti-HLA-DQ) there was no observable inhibition of staining when the KC were pre-incubated with TS 1·16, suggesting that the Leu 10 antibody was not binding to HLA-DR antigen (data not shown). This was also confirmed by the difference in the intensity of cell-surface staining between HLA-DR and HLA-DQ.

Effect of serum-supplemented medium on the IFN-γ-induced expression of HLA-DR, HLA-DQ and ICAM-1

All three antigens were expressed less strongly on KC cultured DMEM + 10% FCS than on cells cultured in KGM. The cell surface expression of ICAM-1 and HLA-DR was similar to that seen on KC cultured in KGM, but the intracellular staining was markedly reduced (data

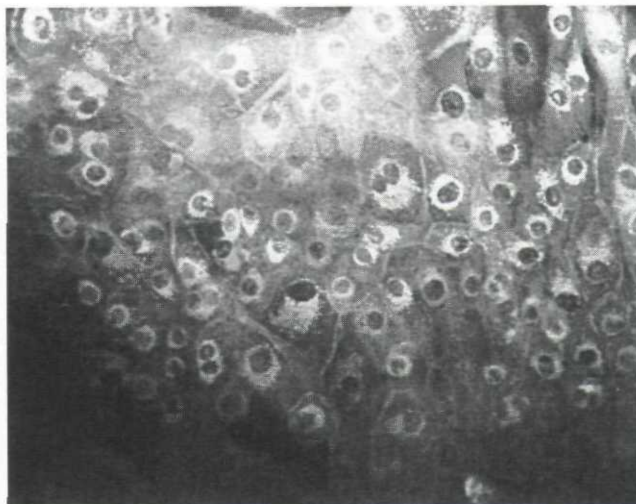

FIGURE 1. IFN-γ-induced expression of HLA-DR on acetone-fixed keratinocytes showing perinuclear and intense cell surface staining (original × 150).

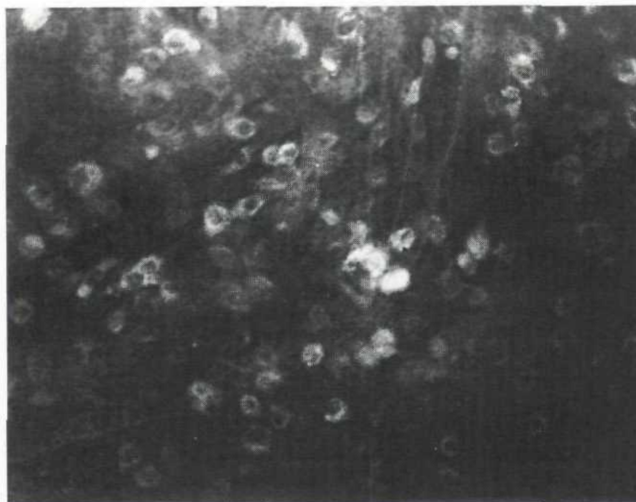

FIGURE 2. IFN-γ-induced expression of HLA-DQ on acetone-fixed keratinocytes showing perinuclear and minimal cell surface staining (original × 150).

not shown). There was no cell surface expression of HLA-DQ on KC cultured in DMEM + 10% FCS and the intracellular staining was also markedly reduced.

Intensity of cell surface expression of HLA-DR, HLA-DQ and ICAM-1 antigens using FACS analysis

After subtraction of OKT8 staining (which was used as the IgG_1 isotype control) background values, the mean channel readings for the three antigens studied were: HLA-DQ: 18, HLA-DR: 52 and ICAM-1: 79.

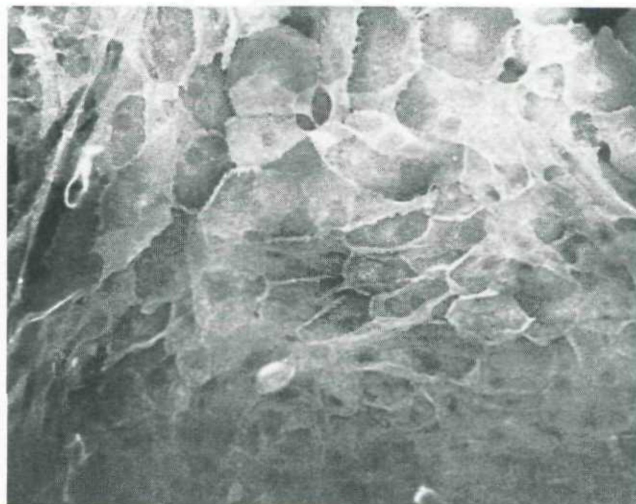

FIGURE 3. IFN-γ-induced expression of ICAM-1 on acetone-fixed keratinocytes showing intense cell surface and diffuse cytoplasmic staining (original × 150).

These values are for KC grown in KGM supplemented with IFN-γ (1200 U/ml) for 3 days. In the absence of IFN-γ, the mean channel readings were 0 for all three antigens, as well as for the OKT8 stained KC. It is important to use isotype controls because we have previously observed that IFN-γ induces expression of Fc receptors in cultured KC.[19] It should also be noted that the mean channel readings presented are on a log scale; thus the values represent a considerable degree of fluorescence intensity.

DISCUSSION

This study has demonstrated the ability of IFN-γ to induce contrasting and distinctive cell surface and intracellular expression of HLA-DR, HLA-DQ and ICAM-1 antigens in keratinocytes. The expression of these antigens was diminished by growing the KC in medium supplemented with serum. The induction of HLA-DQ by IFN-γ is of interest as Basham *et al.*[19] in a previous study, were unable to demonstrate this antigen on normal human KC. This disparity can be explained by the fact that they cultured KC in DMEM + 10% FCS which we have shown to abrogate the cell surface expression of HLA-DQ. The observed reduction in IFN-γ-induced antigen expression in serum-supplemented medium was most probably a result of the greater degree of KC differentiation observed under these culture conditions.

The rarity of HLA-DQ expression on keratinocytes *in vivo* could well be because of the intracellular localization of this molecule which may not be detected by conventional staining techniques. It has been demonstrated that the cell surface expression of HLA-DR is five times greater than that of HLA-DQ,[29] and shedding of the HLA-DQ molecule has also been postulated as a reason for its limited cell surface occurrence.[31] Even in the inflammatory dermatoses, characterized by intense KC expression of HLA-DR, the concomitant appearance of HLA-DQ is unusual, and to date has only been observed in cutaneous T cell lymphoma, lichen planus,[14] *Borrelia*-induced skin disease,[15] and allergic and irritant contact dermatitis.[16] This may be a marker of different KC function in these diseases, but is more probably a result of

higher concentrations of IFN-γ produced by dermal or intra-epidermal activated T lymphocytes in these particular dermatoses. An analogous situation has been observed in auto-immune thyroid disease where in class II antigen expression by thyrocytes HLA-DR is more commonly and intensely expressed than HLA-DQ.[32] The same pattern is seen in cultured thyrocytes treated with IFN-γ.[32] Measurement of steady-state mRNA levels transcribed from different HLA-DQ loci may allow further identification of the genes upon which IFN-γ acts in human KC. Further study of the regulation of HLA-DQ is required since one allele (HLA-DQβ) has recently been found to be associated with pemphigus vulgaris.[33] Furthermore, HLA-DQ expression by lymphoid cells has been associated with antigen-specific suppression of the immune response.[34] Since IFN-γ treated KCs have also been observed to induce antigen-specific unresponsiveness,[35] it will be of interest to determine whether such reactions are mediated via HLA-DQ.

The ability of IFN-γ to induce ICAM-1 expression on the surface of KC and the intense cell surface expression of this antigen is of major importance in the lymphocyte–KC adherence reaction *in vitro*[24,25] and may also be relevant in many inflammatory dermatoses *in vivo*.[20] It has been demonstrated that anti-LFA-1 and anti-ICAM-1 antibodies, but not anti-HLA-DR antibodies, inhibit the binding of T lymphocytes to IFN-γ-treated KC[24-26] thus producing additional evidence that ICAM-1 is involved in this process. The prominent cell-surface expression of this antigen by KC after IFN-γ exposure supports this idea.

Thus, it is postulated that activated T lymphocytes in the dermis induce ICAM-1 expression on KC by means of IFN-γ production. The present work extends our previous suggestion that IFN-γ is the common molecule responsible for the expression of HLA-DR on KC in a variety of inflammatory dermatoses.[36] ICAM-1 expression of KC could promote lymphocyte–KC adherence reactions and the subsequent events leading to class II antigen induction on KC, which may contibute to the pathophysiological changes observed in a variety of inflammatory dermatoses.[37]

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