Interferon Enhancement of HLA-DR Antigen Expression on Epidermal Langerhans Cells

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Langerhans cells (LCs) are dendritic epidermal cells whose ability to function as accessory/stimulatory cells in initiating the immune response is, like that of macrophages, dependent on the expression of class II major histocompatibility antigens. In normal human skin approximately 50% of LCs identified by cell surface T6 antigenicity also express HLA-DR histocompatibility determinants. We report here that recombinant DNA-derived human interferon (IFN)-gamma, but not IFN-alpha2, induces the expression of HLA-DR antigens by the population of human epidermal LCs on which such antigens normally are not detected. IFN-gamma effectively induced HLA-DR on both neonatal and adult epidermal LCs and such induction was blocked by neutralization with a murine monoclonal antibody to IFN-gamma. IFN-gamma induction of LC HLA-DR expression is inhibited by prostaglandin E2 (PGE2) and is mimicked by the presence of fatty acid cyclooxygenase inhibitors, known to reduce PGE2 production. These results suggest that IFN-gamma may play a role in regulating skin-associated immune responses through enhanced expression of HLA-DR antigens on LCs and that such enhancement may be mediated by alterations in arachidonic acid metabolism.

The major histocompatibility complex encodes for two classes of cell surface glycoproteins. In humans these membrane proteins are class I (HLA-A, B, C) and class II (HLA-DR, DS, SB) molecules [1-3]. In contrast to class I antigens which are expressed upon most somatic cells except adult erythrocytes, class II antigens have a restricted cellular distribution. The most extensively studied class II antigen, HLA-DR, for example, is normally expressed only by B cells and accessory cells such as monocytes, macrophages, dendritic cells, and Langerhans cells (LCs) [4-7]. HLA-DR molecules are homologous structurally and functionally with murine Ia surface glycoproteins [8], and are functionally important in that their expression is required for accessory cells to present nominal antigen to helper T cells and initiate the immune response [9-13].

HLA-DR molecules are also important as stimulatory antigens in triggering mixed lymphocyte reactions [9] and mixed epidermal cell-lymphocyte reactions [12,13].

Several studies in both the human and murine systems have indicated that expression of DR/Ia antigens upon B cells and macrophages can be stimulated by soluble products of activated T cells [14-17]. Specifically, recent studies have identified the DR/Ia inducing lymphokine as interferon (IFN)-gamma, and have shown that IFN-gamma produced from the cloned IFN-gamma gene is capable of such DR/Ia induction [18-20]. Both IFN-alpha and IFN-beta appear to be either less effective or ineffective as DR/Ia inducers [21,22].

LCs, which are derived from bone marrow precursor cells [23] and reside within the epidermis, share many characteristics with macrophages inducing DR/Ia expression [6,7] and accessory cell functions [10-13,24]. LCs are the only cells in the normal human epidermis to express DR-antigen and the cell surface antigen T6, a molecule also found on human thymocytes [25]. Just as not all macrophages express DR antigens, using immunofluorescent microscopic techniques, we and others have found that approximately 50% of T6 bearing LCs do not express detectable levels of DR antigens [26-28]. In this study we report a selective enhancement of HLA-DR expression on human epidermal LCs that is effected by exposure to recombinant DNA-derived IFN-gamma, but not to IFN-alpha.

The intracellular mechanisms responsible for IFN-gamma induction of DR/Ia antigens are unknown, but studies with murine macrophages suggest that products of the arachidonic acid cascade are involved as prostaglandins of the E series (PGE) were reported to inhibit lymphokine-induced Ia expression [18,29]. Moreover, the cyclooxygenase inhibitor, indomethacin, mimicked lymphokine action by inducing macrophage Ia expression by itself [29]. Similarly, we report here that PGE2 inhibits the IFN-gamma induced HLA-DR expression on LCs and further demonstrate that indomethacin and 3 other cyclooxygenase inhibitors can mimic IFN-gamma action by increasing HLA-DR expression on LCs.

MATERIALS AND METHODS

Interferons and Anti-Interferons

Human recombinant IFN-alpha, (6.6 × 10^7 units/ml) from Escherichia coli was kindly supplied by Schering Corporation, Kenilworth, New Jersey. Human recombinant E. coli-derived IFN-gamma (1.0 × 10^7 units/ml) and murine monoclonal antibody (IgG1) to human IFN-gamma (5 × 10^4 neutralizing units/ml) were kindly supplied by Genentech, Inc., South San Francisco, California.

Chemicals

PGE2, thromboxane B2 (TXB2), indomethacin, nordihydroguaiaretic acid (NDGA), and imidazole were obtained from Sigma Chemical, St. Louis, Missouri. Benoxaprofen and 5,8,11,14-eicosatetraynoic acid (ETYA) were obtained from Eli Lilly, Inc., Indianapolis, Indiana and Hoffmann-LaRoche, Inc., Nutley, New Jersey. PGE2, TXB2, and indomethacin were dissolved in absolute ethanol and diluted in RPMI-1640 heat-inactivated fetal calf serum (ΔFCS) to the final tested concentrations. At the dilutions tested ethanol had no effect on HLA-
DR expression. Benoxaprofen, ETYA, and NDGA were dissolved in minimal amounts of 1 N NaOH, immediately neutralized with 1 N HCl, and diluted (as was imidazole directly) in RPMI-10% ΔFCS to the final concentrations desired.

**Dispersed Epidermal Cell Cultures**

Dispersed epidermal cells (5 × 10⁶) isolated from neonatal human foreskin as described in [7], were maintained in vitro at 37°C for 20 h in humidified 5% CO₂ in 2.0 ml RPMI-1640 containing 10% ΔFCS and the indicated concentrations of either pure, E. coli recombinant DNA derived human IFN-α, IFN-γ, or other compounds as indicated. Dispersed epidermal cells from adult facial skin were isolated and maintained as indicated above in 3 separate experiments.

**Detection of LC Surface Antigens HLA-DR and T6 on Dispersed Epidermal Cells**

Simultaneous detection of HLA-DR and T6 antigen-bearing cells was achieved under epifluorescent microscopy following sequential treatment of the epidermal cells with mouse monoclonal anti-HLA-DR IgG, (Ortho Diagnostics, Raritan, New Jersey), rhodaminated goat antimouse IgG (Meloy Labs, Springfield, Virginia), mouse monoclonal anti-T1 IgG (to saturate free antimouse IgG binding sites), and fluoresceinated mouse monoclonal anti-T6 IgG (Ortho Diagnostics) [26]. Epidermal cells were examined as wet mounts under epifluorescent microscopy with appropriate excitatory and barrier filters and the mean number, and SD, of DR⁺/T6⁺ cells were calculated.

**Skin Explant Incubation In Vitro**

Four millimeter-diameter specimens of freshly excised human neonatal foreskin, trimmed of fat and dermis, were maintained in vitro under conditions identical to those described for dispersed epidermal cells. After 20 h in culture, intact epidermal sheets were prepared from each specimen following incubation in 1 N NaBr at 37°C for 60 min. Adult facial skin prepared and maintained under these conditions was utilized in 2 separate experiments.

**Detection of HLA-DR Bearing LC in Epidermal Sheets**

Epidermal sheets were incubated with either mouse monoclonal anti-HLA-DR IgG or anti-T6 IgG (Ortho Diagnostics) followed by incubation with fluoresceinated goat antimouse IgG (Meloy Labs), at 23°C [26]. The number of fluorescing, dendritic cells in ten 400 × fields was counted under epifluorescent microscopy and a mean and SEM per mm² calculated.

**Statistics**

The statistical difference between means was calculated using the Student's one-tailed t-test with probability values less than 0.01 being considered significant.

**RESULTS**

**Effect of Interferons on Expression of HLA-DR Antigens by Dispersed Epidermal Cells**

To determine their effects on surface HLA-DR expression, E. coli-derived IFN-α and IFN-γ were tested initially on cultured neonatal human epidermal cells. LCs constitute 2-5% of human epidermal cells [30] and, within the epidermis, may be specifically identified by the presence of cell surface T6 antigen [25]. We [26] and others [27,28] have reported that only half of T6-positive LCs also express HLA-DR antigens. As shown in Table I, the addition of IFN-gamma to human epidermal cell cultures increased the number of DR⁺/T6⁺ LCs detected, even at the lowest concentration (10 units/ml) tested. The number of DR⁺ cells approached, but did not exceed, the constant (2.40 × 10⁶) total number of simultaneously detected T6 antigen-bearing LCs. No enhancement of DR-bearing LCs was detected following the addition of IFN-alpha, even at 10⁴ units/ml.

The IFN-gamma enhancement of DR-bearing LCs was not restricted to neonatal epidermal cells but also occurred when adult epidermal cells were studied. In 3 experiments utilizing adult face skin epidermal cell cultures, incubation with 10⁴ units/ml of IFN-gamma was found to double the number of epidermal cells expressing HLA-DR antigens from 1.08% ± 0.20 (SEM) of all epidermal cells in control cultures to 2.00% ± 0.48 after 20 h of incubation with IFN-gamma.

**Effect of Interferons on Expression of HLA-DR Antigens on LCs in Situ**

To further approximate in vivo conditions and to avoid enzymatic treatments and other physical manipulations required to isolate and disperse epidermal cells for culturing [7], superficial specimens of human foreskin were maintained in culture for 20 h in the absence and presence of various concentrations of IFN-gamma and IFN-alpha. Following treatment of the specimens with NaBr, the number of DR and T6 antigen-bearing cells per mm² in the resultant epidermal sheets was calculated. As shown in Table II, exposure to IFN-gamma (10¹-10⁶ units/ml) enhanced the detection of DR antigen-bearing epidermal cells with no increases being detected following the addition of identical concentrations of IFN-alpha. All DR-bearing cells were dendritic, and T6 antigen-bearing LCs were not increased in number.

We found this in vitro method utilizing intact skin specimens to be a simple and reproducible technique for studying LC surface antigens. As detailed in Table III, a series of 9 such in situ experiments using 20 h of foreskin exposure to 10⁶ units/ml IFN-gamma reproducibly increased the density of HLA-DR

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Units/ml</th>
<th>HLA-DR⁺ Cells/mm² (SEM)</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>267.3 (25.6)</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>IFN-alpha₂</td>
<td>10¹</td>
<td>241.5 (16.1)</td>
<td>90.3</td>
</tr>
<tr>
<td></td>
<td>10²</td>
<td>257.6 (19.3)</td>
<td>96.3</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>265.6 (20.9)</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>209.3 (14.5)</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
<td>276.9 (22.5)</td>
<td>103.5</td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>10¹</td>
<td>360.1 (17.7)</td>
<td>115.6</td>
</tr>
<tr>
<td></td>
<td>10²</td>
<td>383.2 (29.0)</td>
<td>143.³</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>544.2 (29.0)</td>
<td>203.5</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>520.0 (38.6)</td>
<td>194.5</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
<td>566.7 (40.3)</td>
<td>212.0</td>
</tr>
</tbody>
</table>

*Four millimeter-diameter specimens of freshly excised human neonatal foreskin, trimmed of fat and dermis, were maintained in vitro as described in Materials and Methods.

**TABLE I. Expression of Langerhans cell HLA-DR antigens following exposure of dispersed epidermal cells to IFN-alpha₂ and IFN-gamma**

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**TABLE II. Effect of IFN-alpha₂ and IFN-gamma on expression of HLA-DR antigens on Langerhans cells in situ**

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**TABLE III. Effect of IFN-gamma on expression of HLA-DR antigens on LCs in situ**

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**DISCUSSION**

The enhanced detection of DR⁺ cells in situ may be due to physical differences in the epidermal sheets compared to cultured cells, as well as factors unique to the in vivo environment. The enhanced detection of DR⁺ cells in situ may be due to physical differences in the epidermal sheets compared to cultured cells, as well as factors unique to the in vivo environment. The enhanced detection of DR⁺ cells in situ may be due to physical differences in the epidermal sheets compared to cultured cells, as well as factors unique to the in vivo environment.
TABLE III. IFN-gamma enhancement of Langerhans cell HLA-DR antigenicity in situ

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>IFN-gamma</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>313.9 (16.1)</td>
<td>529.6 (25.7)</td>
<td>168.7</td>
</tr>
<tr>
<td>2</td>
<td>319.5 (10.0)</td>
<td>496.7 (16.9)</td>
<td>155.4</td>
</tr>
<tr>
<td>3</td>
<td>294.6 (26.8)</td>
<td>513.5 (36.5)</td>
<td>174.3</td>
</tr>
<tr>
<td>4</td>
<td>322.0 (10.4)</td>
<td>604.5 (35.4)</td>
<td>187.7</td>
</tr>
<tr>
<td>5</td>
<td>267.3 (25.6)</td>
<td>544.2 (29.0)</td>
<td>205.5</td>
</tr>
<tr>
<td>6</td>
<td>273.7 (15.0)</td>
<td>500.7 (12.1)</td>
<td>182.9</td>
</tr>
<tr>
<td>7</td>
<td>314.0 (11.1)</td>
<td>567.3 (14.5)</td>
<td>180.6</td>
</tr>
<tr>
<td>8</td>
<td>286.6 (9.7)</td>
<td>518.4 (22.5)</td>
<td>180.8</td>
</tr>
<tr>
<td>9</td>
<td>342.9 (12.7)</td>
<td>620.1 (17.2)</td>
<td>181.7</td>
</tr>
</tbody>
</table>

Mean: 177.9 (4.3)

* Nine separate experiments were carried out in which human foreskin specimens were prepared, cultured, and analyzed for epidermal HLA-DR antigenicity as described in Materials and Methods, employing 10^6 units/ml IFN-gamma.

* Each value listed in this column is significantly different (p < 0.01) from its corresponding untreated control value.

antigen-bearing LCs over control specimens by 77.9% ± 4.3 (SEM). Extending the duration of incubation with IFN-gamma to 48 h did not result in any further increases in HLA-DR+ LCs.

As observed with dispersed epidermal cell cultures, IFN-gamma also enhanced the expression of HLA-DR by LCs in situ in adult skin specimens. In 2 separate experiments using specimens of adult face skin, incubation with 10^6 units IFN-gamma increased the number of in situ DR+ cells/mm^2 by an average of 80% over controls.

Inhibition of IFN-gamma Enhancement of LC HLA-DR Expression by Monoclonal Anti-IFN-gamma

To be certain that cloned IFN-gamma itself and not some other E. coli-derived contaminant possibly present in our IFN-gamma preparation was inducing HLA-DR expression, we studied IFN-gamma induced DR enhancement in the presence of a monoclonal anti-IFN-gamma. The addition of 100 units/ml IFN-gamma to intact foreskin specimens significantly (p < 0.01) increased the percentage of HLA-DR+ cells (363.1% ± 3.5) over that present in untreated controls (100.0% ± 2.1). The simultaneous addition of 400 neutralizing units/ml of anti IFN-gamma monoclonal antibody significantly (p < 0.01) prevented (102.2% ± 2.2) HLA-DR expression induced by IFN-gamma. The percentage of HLA-DR+ cells in specimens treated with this antibody alone did not differ significantly from untreated control specimens. The presence of a mouse monoclonal anti-IFN-alpha antibody did not significantly prevent IFN-gamma induced HLA-DR expression nor did it affect the number of HLA-DR+ cells in control specimens.

Inhibition of IFN-gamma Induced HLA-DR Expression on LCs by PGE,

PGE1 and PGE2, both products of fatty acid cyclooxygenase action on arachidonic acid, inhibit lymphokine-induced murine macrophage expression of Ia antigens [18,29]. These findings prompted us to determine whether PGE2 could inhibit optimal induction by IFN-gamma of HLA-DR expression on LCs contained in cultured foreskin specimens. As shown in Table IV, 10^-7 M PGE2 effectively inhibited the HLA-DR expression induced by 10^6 units IFN-gamma/ml, while TXB2 had no such inhibitory activity. This observation suggests that IFN-gamma may be inducing HLA-DR expression on LCs by inhibiting the synthesis of PGE2.

Enhancement of LC HLA-DR Antigenicity by Fatty Acid Cyclooxygenase Inhibitors

In order to demonstrate that inhibition of PGE2 synthesis can result in enhanced expression of HLA-DR antigen on LCs, we investigated whether compounds inhibiting PGE2 synthesis via inhibition of fatty acid cyclooxygenase could in the absence of exogenously added IFN-gamma increase HLA-DR expression. Foreskin specimens were maintained either in RPMI alone or in RPMI containing IFN-gamma at 10^9 units/ml, in the presence or absence of known inhibitory concentrations of indomethacin, a specific cyclooxygenase inhibitor [31] or benoxaprofen, ETYA, and NDGA, compounds which inhibit both the cyclooxygenase and lipoxygenase metabolism of arachidonic acid [32-34]. As shown in Fig 1, all these inhibitors significantly increased the number of HLA-DR antigen-bearing epidermal LCs, with indomethacin, ETYA, and NDGA approaching values induced by IFN-gamma. Thus, known inhib-

TABLE IV. Effect of prostaglandin E2 and thromboxane B2 on IFN-gamma induced HLA-DR expression on Langerhans cells in situ

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HLA-DR+ Langerhans</th>
<th>Cells % Control (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100.0 (3.9)</td>
<td></td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>162.7 (5.1)</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>102.8 (4.3)</td>
<td></td>
</tr>
<tr>
<td>IFN-Gamma + PGE2</td>
<td>123.2 (5.1)*</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100.0 (5.9)</td>
<td></td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>172.1 (7.6)</td>
<td></td>
</tr>
<tr>
<td>TXB2</td>
<td>101.9 (7.8)</td>
<td></td>
</tr>
<tr>
<td>IFN-gamma + TXB2</td>
<td>157.3 (4.3)*</td>
<td></td>
</tr>
</tbody>
</table>

* Human foreskin specimens were prepared, cultured, and analyzed for epidermal HLA-DR antigenicity as described in Materials and Methods and the number of HLA-DR+ LCs expressed as a percentage of those present in the untreated control.

* Results are the mean of 9 separate experiments employing 10^6 units/ml IFN-gamma and 10^-7 M PGE2.

* Significantly different (p < 0.01) from value for IFN-gamma treatment alone.

* Results are the mean of 5 separate experiments employing 10^3 units/ml IFN-gamma and 10^-8 M TXB2.

* Does not significantly differ (p > 0.01) from value for IFN-gamma treatment alone.

FIG 1. Effect of arachidonate cyclooxygenase inhibitors on the expression of Langerhans cell HLA-DR antigenicity in situ.

Superficial foreskin specimens were prepared and maintained as described in Materials and Methods in the absence or presence of the compounds indicated. The bars represent the mean ± SEM of 16 separate experiments for controls, 8 experiments for indomethacin (10^-5 M), 6 experiments for benoxaprofen (10^-4 M), 3 experiments for ETYA (10^-5 M), 4 experiments for NDGA (10^-5 M), and 3 experiments for imidazole (10^-4 M). Cross-hatched bars indicate the presence of 10^9 units/ml IFN-gamma and open bars indicate its absence. Results are shown as the number of HLA-DR+ cells expressed as a percentage of those present in untreated controls. All treatments significantly (p < 0.01) increased HLA-DR+ cells over untreated controls with the exception of the addition of imidazole alone.
interleukin-18, a cytokine that activates macrophages and T cells, enhances the expression of HLA-DR on LCs [40, 41]. LC accessory cell functions are in all likelihood active in contact hypersensitivity, skin graft rejection and certain types of drug hypersensitivities. We are presently examining whether the induction of HLA-DR antigens on LCs by IFN-gamma results in an increase in LC accessory/stimulatory cell functions.

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REFERENCES