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Gamma Interferon Modulates Epidermal Cell Proliferation and Mixed Epidermal Cell-Lymphocyte Reaction

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Gamma interferon (γ-IFN), a lymphokine produced by activated T lymphocytes, has a variety of effects on target cell. It induces class II antigens of the major histocompatibility complex not only in immunocompetent cells but also in non-immunocompetent cells. γ-IFN also can exert, in addition to anti-viral activity, a series of anticellular effects on a variety of cell types.

The effects of γ-IFN on the proliferation of cultured epidermal cell (EC) and induction of HLA-DR antigen expression by EC (HLA-DR$^+$KC) were studied. Furthermore, the immunologic role of HLA-DR$^+$KC in the mixed epidermal cell-lymphocyte reaction (MECLR) was studied.

The antiproliferative effect of γ-IFN on the cultured EC was seen 3 days after treatment of γ - IFN and the effect was dose-dependent. Number of HLA-DR$^+$KC was increased dose-dependently with treatment of γ-IFN. In MECLR, HLA-DR$^+$KC had been found to exert stimulatory role on allogenic lymphocytes. However, there was no significant role of HLA-DR$^+$KC on autologous lymphocytes.

Key Words: *Epidermal cell (EC), Gamma-interferon (γ-IFN) HLA-DR positive keratinocyte (HLA-DR$^+$KC), Keratinocyte (KC), Langerhans cell (LC), Mixed epidermal cell-lymphocyte reaction (MECLR) Peripheral blood lymphocytes (PBL).*

INTRODUCTION

Human major histocompatibility complex (MHC) class II antigens are generally considered to play an important role in antigen presentation and various cellular interactions that are required during the immune responses.

In normal murine and human epidermis, the expression of class II antigens are restricted to bone marrow-derived Langerhans cells (LC). Recently several investigators have reported on the detection of class II antigens on keratinocytes (KC) in certain disease states, e.g. cutaneous T cell lymphoma, lichen planus, allergic contact dermatitis and cutaneous graft-versus-host disease (Tjernlund, 1978; Tjernlund, 1980; Lampert et al., 1982, Mackie &

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Turbitt, 1983). However, whether the expression of HLA-DR antigens found on KC (HLA-DR^+KC) actively serve a role in the cell interactions in immune system remains to be clarified.

Robert et al. (1985a) have reported that one possible role of the HLA-DR^+KC is to facilitate the movement of LC into the epidermis. In their other study (1985b) suggested that a well correlation between Ia expression by KC and the intensity and duration of contact hypersensitivity might be due to either through some signaling process or their ability to function as tissue localizing antigen presenting cells.

By the report of Nickoloff et al. (1986) on human KC-lymphocyte interaction, HLA-DR^+KC did suppressive role on allogenic lymphocytes, but some cases showed significant stimulation on allogenic lymphocytes.

Gamma interferon was found to be capable of inducing class II antigens of the MHC not only in immunocompetent cells such as monocyte but also in nonimmunocompetent cells of endothelial cells, melanocytes and KC (Pober et al., 1983; Basham et al., 1984; Nickoloff et al., 1986). In addition, γ-IFN has shown to inhibit viral proliferation in number of cell types and also inhibit the growth of normal KC and squamous cell carcinoma cells in vitro (Nickoloff et al., 1984; Nickoloff et al., 1985).

In an attempt to better understand HLA-DR expression by KC, the effects of the natural form of r-IFN on the growth and expression of HLA-DR antigen by cultured epidermal cells (EC) were investigated. Furthermore, the immunologic role of HLA-DR^+KC in the mixed epidermal cell-lymphocyte reaction (MECLR) was studied.

MATERIALS AND METHODS

1. Epidermal cell sampling

Skin specimens were obtained from dermatologically normal persons, nineteen men and six women aged 18 and 38 years.

The method of Briggaman et al. (1967) was modified to prepare EC suspension. Briefly, the majority of dermis was cut off and the resulting split-thickness sections were placed overnight 4°C in Hank's balanced salt solution (HBSS) containing 0.25% trypsin, 0.1% EDTA. The epidermis was peeled off and the dermis side was agitated gently in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100ug/ml). Separated ECs were dispersed by gentle suction through Pasteur pipette and counted in a hemocytometer.

Viability of KC suspension as determined by trypan blue dye exclusion was more than 90%. EC was confirmed by inverted microscope with direct observation. Further confirmation was made with electron microscope and stained with monoclonal antikeratin antibodies, provided by Dr. Tung-Tien Sun, Department of dermatology, New York University School of Medicine, New York (Sun & Green, 1978).

2. Peripheral blood lymphocyte (PBL) isolation

PBL was obtained by the modified method of Terasaki & McClelland (1964). Briefly, heparinized venous blood from skin donors was centrifuged at 1,000xg for ten min. One ml of buffy coat was collected and mixed with equal volume of HBSS, pH 7.2. Thereafter, it was layered on Ficoll-Hypaque mixtures (specific gravity 1,007) and centrifuged at 1,000xg for ten min. Interface cells were collected and resuspended in HBSS. One drop of bovine thrombin (Parke Davis, U.S.A.) was added for the removal of platelets and polymorpholeukocytes. Lymphocytes were adjusted to 1.0×10^6 cells/ml in Rosewell Park Memorial 1640 (RPMI) containing heat inactivated 10% FBS and antibiotics (penicillin, 100 u/ml and streptomycin, 100 μg/ml).

3. HLA-DR typing

HLA-DR typing was performed by Park & Terasaki method (1979). Briefly, 1 μl of lymphocyte suspension, adjusted to 2.0×10^5 cells/ml, was inoculated in each well of HLA-DR tray (Department of microbiology, Catholic University Medical College, Seoul) and incubated for 30 min at 37°C. Complement (Peel-Freeze, Biologicals, AR., U.S.A.) 5 μl was added and further incubated for 2 hrs at 25°C. When number of dead cells exceed 60%, the well was considered positive.

4. HLA-DR antigen staining of cultured epidermal cells

One ml of EC suspension, adjusted to 5×10^5 cells/ml, was inoculated in each well of 24-well tissue culture clusters (Costar 205 Broadway/ Cambridge, MA., U.S.A.), and the cell culture was maintained in a humidified incubator with 5% CO_2/95% air at 37°C.

Natural γ-IFN (Human gamma interferon; Hu γ-IFN Lot 55F-0685 Sigma, St. Louis, MO., U.S.A.) was added 3 days after cell seeding and thereafter every third days in an amount of 100 u/ml, 300 u/ml, 500 u/m, and 700 u/ml.

The modified method of Leung et al. (1981) was

used for indirect-immunofluorescent staining for HLA-DR antigen staining for EC.

On drop of EC suspension, adjusted to 2×10^5 cells/ml, was put on a slide and fixed with cold acetone at 4°C for 1 min. Slides was washed in PBS and incubated 30 min in a 1:45 dilution of fluorescein-isothiocyanate conjugated goat antimouse IgG. Slide was washed again in PBS, and read under on Olympus epifluorescene microscope and cells showing characteristic granular or rim-like fluorescence were considered as positive. The percentage of EC staining for HLA-DR antigen in a slide was counted in 10 high-power (40x) fields.

5. Mixed epidermal cell-lymphocyte reaction (MECLR)

One hundred μl of EC suspension, adjusted to 5×10^5 cells/ml, per well of 96-flat bottomed tissue culture clusters for 3 days. γ-IFN 300 u/ml was added in day 3 each well and cultured for another 3 days. On day 6 EC was treated with 10 μg/ml of mitomycin for 2 hrs in a dark room. One hundred μl of lymphocyte suspension, adusted to 10×10^6 cells/ml, was put in each well, and incubated for 3 days more.

MECLR was divided into 3 experiment group, that is HLA-DR unmatched (allogenic MECLR), HLA-DR matched (autologous MECLR) and HLA-DR untyped group.

One μCi ^{3}H-thymidine (New England Nuclear, Boston, MA, U.S.A.) per well added in the culture of epidermal cell-lymphocyte and 18 hrs later, cells harvested and counted the uptake of ^{3}H-thymidine (cpm).

RESULTS

1. Antiproliferative effect of γ-IFN on epidermal cell

The antiproliferative effect of γ-IFN was already shown on day 3 (Table 1), 3 days after the γ-IFN treatment of EC. The average number of EC treated with γ-IFN 100 u/ml on day 3 was 1.55±0.70 ($\times10^5$). When γ-IFN concentration was increased to 300 u/ml, 500 u/ml and 700 u/ml, the numbers of EC was 1.20±0.45 ($\times10^5$), 0.98±0.45 ($\times10^5$) and 0.74±0.47 ($\times10^5$) respectively. The average number of EC in a well which was treated with γ-IFN 100 u/ml on day 12 was 1.76±0.62 ($\times10^5$). when γ-IFN was increased to 300 u/ml, 500 u/ml and 700 u/ml on day 12, the cell

Table 2. HLA-DR$^+$KC induction by γ-IFN treatment (%)

γ-IFN	Duration of treatment	
	3 days	6 days
untreated (Contorl)	1.68±0.20	1.27±0.26
100	12.60±2.87	9.37±2.10
300	22.60±8.03	18.50±0.42
500	30.12±3.21	23.11±3.06
700	28.47±5.26	26.70±1.94

* HLA-DR$^+$KC: HLA-DR antigen positive Keratinocyte.
Inoculum size: $5+10^5$ EC on each well of 24-well tissue culture culster.
The rate of HLA-DR$^+$KC by fresh trypsinized EC was 3.81±1.32%.
Each data consists of mean number of 3 experiments made with EC sampled from 3 individuals.

Table 1. Antiproliferative effect of γ-IFN on epidermal cell ($\times10^5$)

Day*	γ-IFN (u/ml)				
	untreated	100	300	500	700
0**	0.95±0.23	1.23±0.76	1.22±0.67	0.93±0.41	0.96±0.31
3	1.65±0.61	1.55±0.70	1.20±0.45	0.98±0.45	0.74±0.47
6	0.26±0.48	1.59±0.59	1.09±0.40	0.90±0.38	0.86±0.36
9	2.27±0.58	1.77±0.67	1.13±0.35	0.76±0.40	0.61±0.27
12	2.36±0.62	1.76±0.62	1.12±0.29	0.64±0.29	0.35±0.14

Inoculum size: 5×10^5 EC on each well of 24- well tissue culture cluster.
* Duration of r-IFN treatment, and γ-IFN was added on days 0,3,6, and 9.
** Day o stand for the third days after the cell seeding.
Mean number of attached EC in a well counted 1 day after the cell seeding was 0.73±0.03 ($\times10^5$).
Each data consists of mean number of 3 experiments made with EC sampled from 3 individuals.

numbers were 1.12±0.29 (x10^5), 0.64±0.29 (x10^5) and 0.35±0.14 (x10^5) respectively.

2. Effects of γ-IFN on HLA-DR antigen expression by epidermal cells (Fig. 1-3)

HLA-DR antigen induction by γ-IFN treated EC was expressed in percent of positive cells (Table 2).

Three days after γ-IFN 100 u/ml treatment, the rate of HLA-DR$^+$KC were 12.60±2.87%. As the dose of γ-IFN was increased to 300 u/ml, 500 u/ml and 700 u/ml, the rate of HLA-DR$^+$KC were 22.60±8.03%, 30.12±3.21% and 28.47±5.26% respectively comparing to 1.68±0.20% of untreated control.

Duration of γ-IFN treatment, 3 days versus 6 days, made little difference in the rate of HLA-DR$^+$KC. However, there was dose-response relationship between γ-IFN and the rate of HLA-DR$^+$KC induction.

3. Effect of γ-IFN treated epidermal cell on mixed epidermal cell-lymphocyte reaction (MECLR)

Considering the optimal antiproliferative effect and induction of HLA-DR$^+$KC by γ-IFN, MECLR was performed after γ-IFN 300 u/ml treatment for 3 days on cultured EC, and compared with untreated control (Table 3).

Table 3. Effect of γ-IFN treated epidermal cell on mixed epidermal cell-lymphocyte reaction (MECLR)

HLA-DR type	MECLR (cpm uptake)	
	γ-IFN (300 u/ml) Treated	untreated
unmatched (n=15)	2095±860*	755±213
Matched (n=11)	1366±733	841±380
untyped (n=13)	1508±804*	865±525

MECLR was performed with EC which was treated with γ-IFN for 3 days after the optimal incubation period was determined by the preliminary allogeneic MECLR with γ-IFN 300 u/ml treted EC, sampled from 3 individuals, either incubated for 3 days or 6 days. The results were 2937±412 in 3 days incubation and 2753±787 in 6 days.

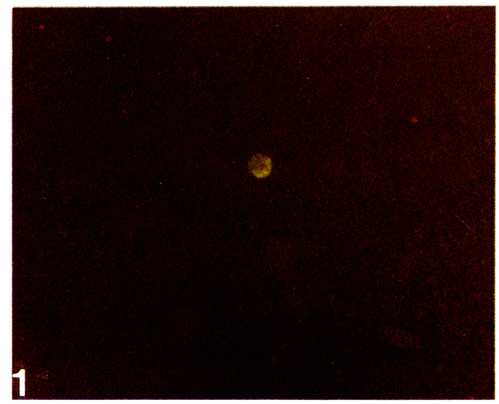
The mean ^{3}H-thymidine uptake of EC only were approximately 100 whether γ-IFN treated or not.

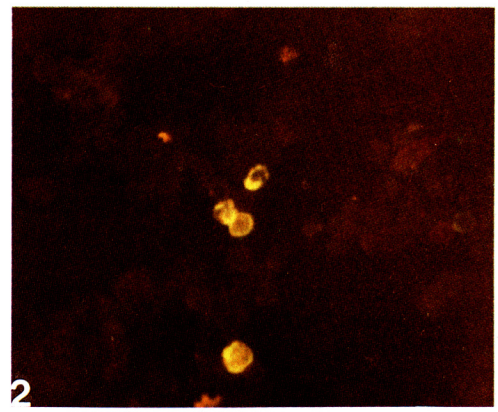
^{3}H-thymidine incorporation of autologous lymphocytes which reacted with γ-IFN treated EC and untreated EC were 649±401 and 502±285.

^{3}H-thymidine incorporation of the responder lymphocyte only with out feeder was 584±204 in autologous group, and the lymphocyte only in unmatched, matched and untyped groups were 578±149, 710±218 and 557-167 respectively.

HLA-DR matched group consists of 4 cases of HLA-DR8, 5 HLA-DR4 and 2 HLA-DR3.

Statistical analysis was used Multiple comparison (Scheffe's method). *$p<0.01$





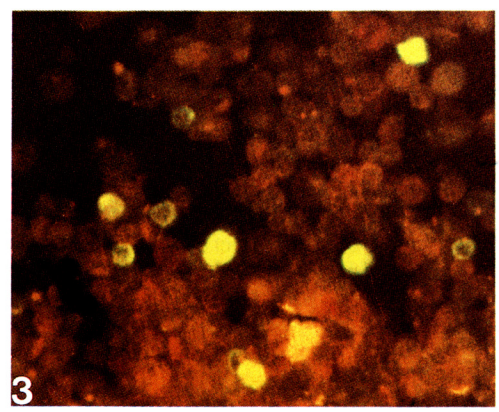


Fig. 1. Immunofluroscent staining of Epidermal cell with anti-HLA DR monoclonal antibody (OKDR) without γ-IFN tratment (control) (x200).

Fig. 2. Immunofluroscent staining of epidermal cell with anti-HLA DR monoclonal antibody (OKDR) after γ-IFN 300 u/ml treatment for 3 days (x200).

Fig. 3. Immunofluroscent staining of epidermal cell with anti-HLA DR monoclonal antibody (OKDR) after γ-IFN 700 u/ml treatment for 3 days (x200).

The mean of ^{3}H-thymidine incorporation in HLA-DR antigen unmatched PBL (allogeneic MECLR) reacted with γ-IFN treated EC and untreated control were 2095±860 and 755±213 respectively.

The mean of ^{3}H-thymidine incorporation of HLA-DR antigen matched PBL (autologous MECLR) reacted with γ-IFN treated EC and untreated control were 1366±733 and 841±380 respectively.

The mean of ^{3}H-thymidine incorporation of HLA-DR untyped PBL reacted with γ-IFN treated EC and untreated control were 1508±804 and 865±525 respectively.

DISCUSSION

Nickoloff et al. (1984) reported antiproliferative effects of recombinant γ-IFN on EC proliferation, in which 100 u/ml of r-IFN added fro 6 days resulted in EC decreased to 50% of control. In our experiment, the EC proliferation rate of γ-IFN 100 u/ml treated for 3 days were 26% compare to pre-γ-IFN treatment. However, EC proliferation rate of γ-IFN untreated on day 3 revealed 74% compared to day 0. Eventually, the antiproliferative effect of γ-IFN was increased as the dose of γ-IFN was increased and the duration of treatment was prolonged.

γ-IFN, known as macrophage activating factor, not only induce class II antigens in macrophage also induce HLA-DR antigen expression of cultured EC (Basham et al., 1984; Czernielewski, 1985; Volc-Platzer et al., 1985).

Treating EC with 100 u/ml of recombinant γ-IFN, Basham et al. (1984) demonstrated that more than 60% of EC were HLA-DR positive. Czernielewski (1985) revealed that, using natural human γ-IFN 500 u/ml for 5 days, more than 20% of EC were HLA-DR positive. In our experiment, the EC treated with γ-IFN 300 u/ml for either d day or 6 days induced HLA-DR^{+}KC respectively 22.60±8.03% and 18.05±0.42%.

Demidem et al. (1986) reported that without γ-IFN treatment, HLA-DR^{+}KC on EC suspension prior to the culture was 2.5±0.5% and they were progressively lost their antigenicity as the culture maintained. In the current experiment, we found HLA-DR^{+}KC at third day after the cell seeding was 1.68±20% compare to 3.81±1.32% of fresh trypsinized EC. This discrepancy may resulted from the fact that the LC which has been viable and active at the begining of cultivation may loose their expressio of HLA-DR antigens during the culture and/or LC itself may be progressively disappeared from the EC culture.

It has been reported that EC can stimulates autologous and allogeneic lymphocytes to proliferate in human and animals (Lane et al., 1975; Tanaka & Sakai, 1979; Sontheimer, 1983; Bagot et al., 1986). However, the effect of γ-IFN in MECLR were controversial. Czernielewski (1985) reported that γ-IFN treated EC played suppressive role in MECLR, whereas Nickoloff et al. (1986) showed more significant proliferative repsonse of allogenic lymphocytes than autologous lymphocytes. This discrepancy may be derived from the fact that while Czernielewski (1985) used trypsinized cultured EC, Nickoloff et al. (1986) performed MECLR in which the EC were still attached to the culture plates, since there are lack of stimulations by the single cell suspensions of the cultured EC with was grown and maintained attached on culture plate, and consquently, the results concur somewhat with Nickoloff et al. (1986).

When Bagot et al. (1986) investigated the possible ability of MECLR to detectalloreactivities in HLA-identical mixed lymphocyte reaction (MLR) negative siblings, the recipient's EC can induce proliferative responses of HLA-identical MLR-negative donor's lymphocytes in 55% of pairs tested. Moreover, further evaluation showed that the positivity of the MECLR before the bone marrow-graft was correlated with later appearance of acute and chronic graft-versus-host disease. Thus, there was discrepancy between MLR and MECLR. Taken together, they concluded that EC have shown to be more efficient antigen presenting cells than PBL for in vitro primary proliferative responses.

In our experiment, γ-IFN untreated EC made no significant changes in either autologous or allogeneic MECLR. On the contrary, γ-IFN treated EC made significant stimulation on allogenic lymphocytes to incorporate ^{3}H-thymidine.

The results of the experiment reported here in demonstrated that γ-IFN appeared to associated with antiproliferative effect of EC and stimulatory role on allogenic lymphocytes to incorporate ^{3}H-thymidine when the appropriate signal (s) was provided by HLA-DR antigen armed EC by γ-IFN.

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