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Prostaglandin E_2 Suppressed IL-15-Mediated Human NK Cell Function Through Down-Regulation of Common γ-Chain[1]

Pratibha C. Joshi,[2]* Xinchun Zhou,* Marvin Cuchens,† and Quintus Jones*

NK cell function is regulated by cytokines and certain biochemical mediators in a positive or negative manner. This study was performed to investigate the suppressive effects of PGE_2 on IL-15-activated human NK cell function. Purified NK cells were cultured with 200 ng/ml IL-15 for 2 days in the presence or absence of 10–200 ng/ml PGE_2. PGE_2 significantly suppressed NK cell-mediated cytotoxicity and IFN-γ production at the secretional and the transcriptional levels. We also evaluated the effect of PGE_2 on the IL-15R complex that consists of IL-2Rβ, common γ-chain (γ_c-chain), and a specific chain IL-15Rα. Percentage of positive cells and number of binding sites for γ_c-chain were significantly increased after IL-15 treatment; however, a substantial decrease was observed with PGE_2 cotreatment. In contrast, constitutive expression of IL-2Rβ was significantly decreased after IL-15 treatment, with no change detected in the presence of PGE_2. At the transcriptional level, neither IL-15 nor PGE_2 had significant effects on the expression of β- or γ_c-chains. There was a 3-fold increase in the expression of IL-15Rα at the transcriptional level that peaked at 8 h after IL-15 treatment; however, PGE_2 had no significant effect. Suppression of NK function by PGE_2 was not due to the endogenous production of IL-4, IL-10, or TGF-β_1 by NK cells. These results suggest that down-regulation of surface expression of γ_c-chain on NK cells may be one mechanism through which PGE_2 mediates suppression of IL-15-activated NK cell function. *The Journal of Immunology,* **2001, 166: 885–891.**

Human NK cells are large granular $CD3^-$ $CD16^+$ $CD56^+$ lymphocytes that play an important role in the host immune response against tumors, virally infected cells, and bacterial infections (1). They are able to initiate host responses without clonal expansion and, therefore, can function as part of the early or "innate" immune response to antigenic challenge. In addition to mediating cytotoxicity, NK cells play an important regulatory role in immune responses through production of cytokines (2). IFN-γ is one of the cytokines produced by activated NK cells. It is a potent activator of macrophages and is critical for the early control of many pathogenic organisms.

In recent years the therapeutic potential of cytokine-activated NK cells has been a major focus of cancer research. Cytokines such as IL-2, IL-15, IL-12, and IL-18 enhance NK cell function (cytotoxicity and IFN-γ production) (3–6) and, therefore, have tremendous potential as therapeutic agents for cancer treatment. Recent studies suggest that IL-15 may be a better candidate for immunotherapy of cancer patients or patients with HIV infection because it has the same effects on NK cell function as IL-2, but is much less toxic (7, 8).

Although potentiating immune responses by cytokines is a promising approach, clinical trials to stimulate the immune system of cancer patients were somewhat disappointing. This failure could be due to the fact that large amounts of immunosuppressive mediators such as PGE_2 are secreted into the tumor environment (9, 10). This may be one of several mechanisms by which tumors evade immune recognition. For example, it is known that PGE_2 has profound suppressive effects in vitro on cellular and humoral immune responses (11). PGE_2 is also known to down-regulate the generation of IL-2-activated killer cell activity (12), although its interactions with IL-15 are not known.

IL-15R complex includes a specific α-chain (IL-15Rα) that is different from IL-2Rα (13). It also uses IL-2Rβ and common γ-chain (γ_c-chain)[3] that is used by IL-2, IL-4, IL-7, and IL-9 for binding and signaling (4). We show here that PGE_2 suppressed IL-15-mediated NK cell function and down-regulated surface γ_c-chain expression.

Materials and Methods

Reagents

Fresh buffy coats were obtained from Mississippi Blood Services (Jackson, MS). Anti-CD19-, anti-CD3-, and anti-CD14-coated magnetic beads were purchased from Dynal (Great Lake, NY). Simultest (anti-CD3-FITC and anti-CD16/CD56-PE) and streptavidin-peridinin chlorophyll protein were purchased from Becton Dickinson (San Jose, CA). Biotinylated anti-CD14, anti-CD19-FITC, and PGE_2 were purchased from Sigma (St. Louis, MO). Quantum Simply Cellular microbeads were obtained from Flow Cytometry Standards (San Juan, PR). PE-conjugated anti-IL-2Rβ (Mikβ1) and anti-IL-2Rγ were purchased from PharMingen (San Diego, CA). Human recombinant IL-15, anti-IL-15 Ab, and anti-IL-15Rα Ab were purchased from R&D Systems (Minneapolis, MN). All primers were obtained from Cruachem (Aston, PA).

Purification of NK cells from buffy coats by negative selection

Mononuclear cells were separated from fresh buffy coats by Ficoll-Hypaque density gradient centrifugation. T lymphocytes were removed by rosetting with 2-aminoethylisothiouronium bromide-treated sheep RBC. Monocytes were removed by plastic adherence. B lymphocytes, residual T cells, and monocytes were removed by incubating cells with anti-CD19-, anti-CD3-, and anti-CD14-coated magnetic beads followed by exposure to magnets. Cells were stained with anti-CD3-FITC plus anti-CD16/CD56-PE (Simultest), anti-CD19-FITC; biotinylated anti-CD14 followed by streptavidin peridinin chlorophyll protein, and were analyzed for purity by flow cytometry

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[3] Abbreviations used in this paper: γ_c-chain, common γ-chain; JAK, Janus kinase.

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(FACScan; Becton Dickinson). NK cells routinely contained <1% $CD3^+$ (T cells), <1% $CD19^+$ (B cells), and <2% $CD14^+$ (monocytes).

Cell cultures

Pure NK cells (2×10^5/well) were cultured in RPMI 1640 with antibiotics and 10% FBS in 96-well U-bottom tissue culture plates with or without different concentrations of rIL-15 and PGE_2 for 1–3 days. The supernatants were harvested and stored at −80°C for measuring cytokines by ELISA. Cells were harvested and either stained with appropriate Abs for flow cytometric analysis or stored in guanidium isothiocyanate at −80°C. PGE_2 was dissolved in 95% ethanol and further diluted with RPMI 1640. The final concentration of ethanol had no effect on NK cell function.

ELISA

Concentrations of IFN-γ, IL-4, IL-10, and TGF-β_1 were determined by an ELISA using commercially available Ab pairs and recombinant standards (PharMingen or R&D Systems) according to a protocol established in our laboratory (14). The lower detection limits for these cytokines were as follows: <10 pg/ml (IL-4), <10 pg/ml (IL-10), <25 pg/ml (IFN-γ), and <200 pg/ml (TGF-β_1).

NK cytotoxicity assay

Cytotoxicity mediated by NK cells was determined by ^{51}Cr release assay (15). Target cells (K-562) were labeled with 100 μCi $Na_2{}^{51}CrO_4$ for 2 h, washed three times, and adjusted to 2×10^5 cells/ml. Serially diluted, activated, purified NK cells were incubated with ^{51}Cr-labeled K-562 cells at various E:T ratios in a routine 4-h assay. One hundred microliters of supernatant from each well were counted for ^{51}Cr release using a gamma counter. Spontaneous and maximum release was measured by adding media and 4% Triton X-67, respectively, to target cell wells. The percent lysis was determined as follows: 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release).

Flow cytometric detection and quantitation of receptor expression

Results were presented as the percent of positive cells or mean channel fluorescence. Briefly, the flurochrome-conjugated Ab was titered to ensure saturation of Ab binding sites on cells. The number of binding sites per cell was calculated by using Quantum Simply Cellular microbeads (16), which uses a mixture of beads with different binding capacities for mouse mAbs. These standard beads were run through a flow cytometer each time to establish a calibration curve. Using Quick Cal software (Flow Cytometry Standards, San Juan, Puerto Rico), the binding capacity of the sample cell population was calculated.

RNA extractions, RT-PCR

Total RNA was extracted from pure NK cells using guanidium isothiocyanate method (17). RNA was reverse transcribed followed by PCR using the following primers: for IL-15Rα, 5′-GTCAAGAGCTACAGCTTGTAC-3′ (forward) and 5′-GTGAGCTTTCTCCTGGAG-3′(reverse); for γ_c-chain, 5′-GGGAACCCAGGAGACAGG-3′ (forward) and 5′-AGCGGCTC CGAACACGAAAC-3′ (reverse); and for IL-2Rβ, 5′-GGCTTTTGGCT TCATCATCT-3′ (forward) and 5′-CTTGTCCCTCTCCAGCACTT-3′ (reverse). G3PDH was used as a control. PCR products were separated on a 2% agarose gel containing ethidium bromide. For quantitation, PCR bands on the negative film of gel photographs were scanned and density of the bands was calculated using image analysis software.

Statistics

The data were analyzed by Student's *t* test or ANOVA with Student-Newman-Kuels test for group comparison and were considered statistically significant at a *p* value of <0.05.

Results

Suppression of NK cell function by PGE_2

The effect of PGE_2 on cytotoxicity and IFN-γ production, two important functions of NK cells, was initially assessed. PGE_2 suppressed cytotoxicity of IL-15-activated human NK cells in a dose-dependent manner (Fig. 1*A*) and at different E:T ratios (Fig. 1*B*). Suppression of NK-mediated cytotoxicity was observed at 1 ng/ml. However, in the presence of higher (100–200 ng/ml) amounts, significant suppression was evident.

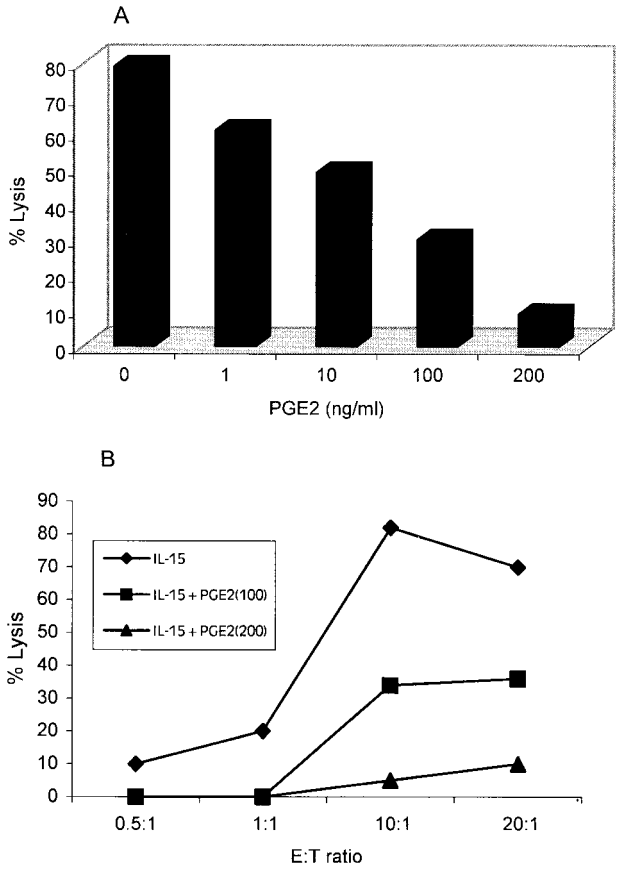

FIGURE 1. Suppression of NK cell function by prostaglandin E_2. Cytotoxicity of IL-15-activated human NK cells was suppressed by PGE_2 in a dose-dependent manner (*A*) and at different E:T ratios (*B*). Purified NK cells were cultured at 2×10^5 cells/200 μl in 96-well plates with 200 ng/ml IL-15 in the presence or absence of different concentrations of PGE_2. Cells were washed after 2 days and 2×10^4 cells/well ^{51}Cr-labeled K-562 cells were added. After incubation at 37°C, 5% CO_2 for 4 h, 100 μl of supernatants from each well were counted. Results are expressed as the percent of lysis from a representative of at least three separate experiments. The SEM was <10%.

IFN-γ production was measured at the secretional level by ELISA and the transcriptional level by RT-PCR. PGE_2 suppressed IFN-γ production at both the protein (Fig. 2*A*) and the mRNA levels (Fig. 2*B*). In day-2 cultures, average IFN-γ production was 12,000 pg/ml (Fig. 1*A*); suppression induced by PGE_2 was dose dependent. IFN-γ secretion was much lower at day 1 (Table I) but increased further in cultures maintained for 5 days, with even greater suppression by PGE_2 (data not shown). At the mRNA level, strong up-regulation of IFN-γ expression was observed at 2 h after IL-15 treatment; IFN-γ expression was not affected by PGE_2. At 18 h, PGE_2-mediated suppression of IFN-γ was more evident. No effect of PGE_2 on the viability, as determined by trypan blue exclusion test, was observed. Likewise, in the presence of IL-15, no significant increase in the number of NK cells was evident.

Expression of IL-15Rα on human NK cells and the effect of PGE_2 on its expression

Surface and mRNA expression of IL-15Rα on NK cells was also examined. Human NK cells expressed constitutive levels of IL-15Rα mRNA; an increase was induced by IL-15, which was dose

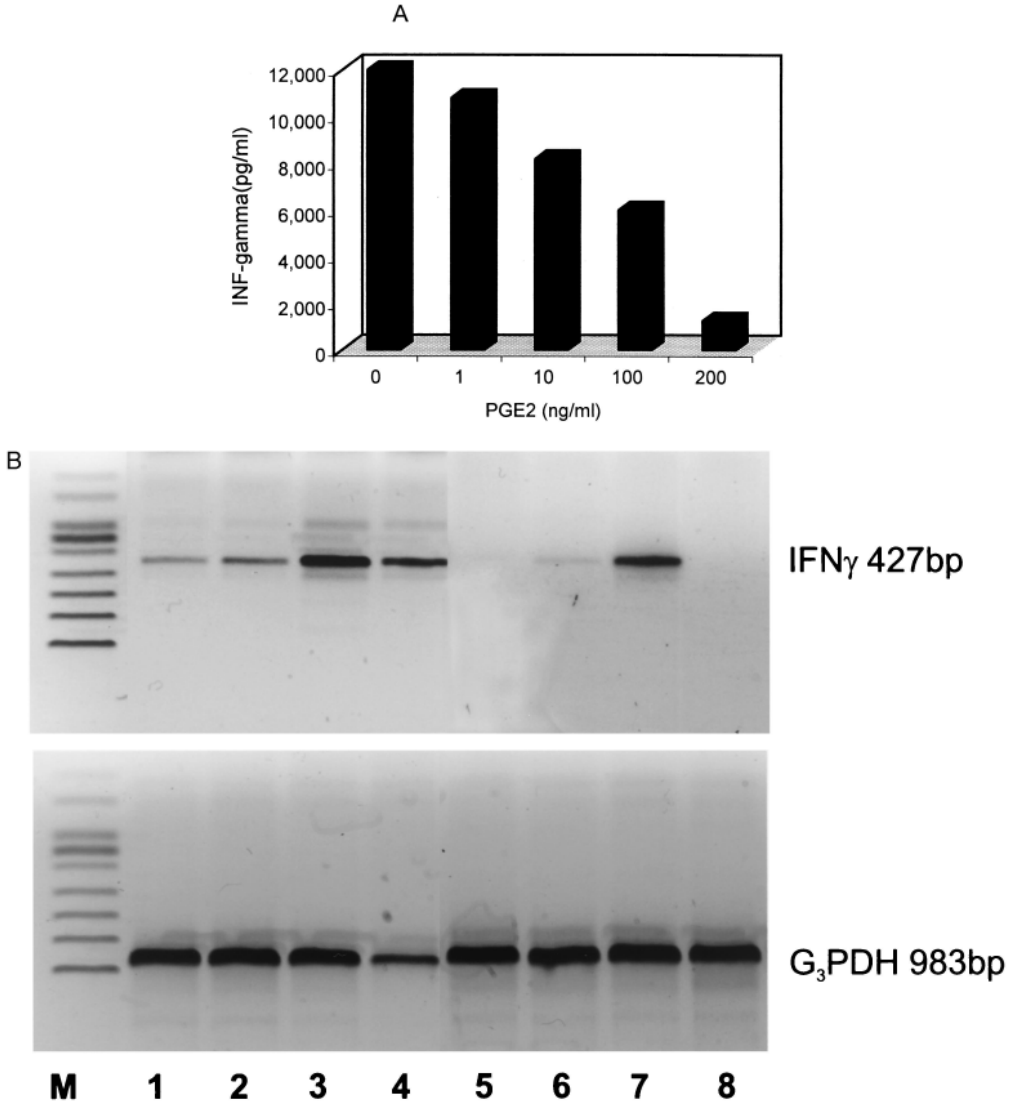

FIGURE 2. IFN-γ production by IL-15-activated human NK cells was suppressed at the secretional level as measured by ELISA (*A*) and at the transcriptional level as measured by RT-PCR (*B*). For ELISA, purified NK cells were cultured at 2×10^5 cells/200 μl in 96-well plates with 200 ng/ml IL-15 in the presence or absence of PGE_2. Supernatants were harvested after 2 days and IFN-γ production was measured. The lower limit of detection was 25 pg/ml. The SEM was <10%. Results are representative of three separate experiments. For RT-PCR, 1×10^6 cells were cultured with medium (1, 5), 200 ng/ml PGE_2 (2, 6), 200 ng/ml IL-15 (3, 7), or IL-15 plus PGE_2 (4, 8) for 2 h (1–4) and 18 h (5–8).

dependent (Fig. 3*A*). This increase was evident at 2 h, peaked at 8 h, and remained elevated at 18 h. Anti-IL-15 Ab inhibited the IL-15-mediated increase in IL-15Rα (see *lane 6*; Fig. 3*A*). As shown in Fig. 3*B*, the density ratio of IL-15Rα/G3PDH was 1.4 for IL-15-treated (18 h) as compared with 0.4 for nontreated cells. PGE_2 did not significantly suppress the IL-15-mediated up-regulation of IL-15Rα. Flow cytometric analysis of surface IL-15Rα expression showed that IL-15Rα was detectable at very low levels and neither IL-15 nor PGE_2 modulated its expression (data not shown).

Table I. *Effect of PGE_2 on INF-γ secretion and surface expressions of IL-2Rβ and γ_c-chain in day-1, day-2, and day-3 NK cell cultures*[a]

				% Positive Cells (no. of binding sites)					
	INF-γ (pg/ml)			IL-2Rβ			γ_c-chain		
Treatment	D1	D2	D3	D1	D2	D3	D1	D2	D3
Medium	ND	ND	ND	93 (2,805)	82 (2,491)	92 (2,601)	28 (1,339)	4 (1,155)	7 (1,116)
PGE_2	ND	ND	ND	89 (3,348)	85 (3,338)	74 (2,477)	7 (930)	4 (1,034)	1 (710)
IL-15	3,314	9,161	18,000	10 (463)	9 (658)	13 (949)	74 (2,730)	52 (2,291)	35 (2,201)
IL-15 + PGE_2	574	ND	ND	26 (837)	25 (1,025)	15 (902)	46 (1,798)	14 (1,275)	6 (1,082)

[a] The detection limit of INF-γ ELISA was 25 pg/ml. Data from a representative experiment is shown. NK cells were cultured with or without 200 ng/ml IL-15 or 200 ng/ml IL-15 plus PGE_2 for 1–3 days as described in *Materials and Methods*. Phenotypic analysis of NK cells before cultures was as follows: 0.7% $CD14^+$, 0% $CD19^+$, 0.3% $CD3^+$, and >90% $CD16^+CD56^+$.

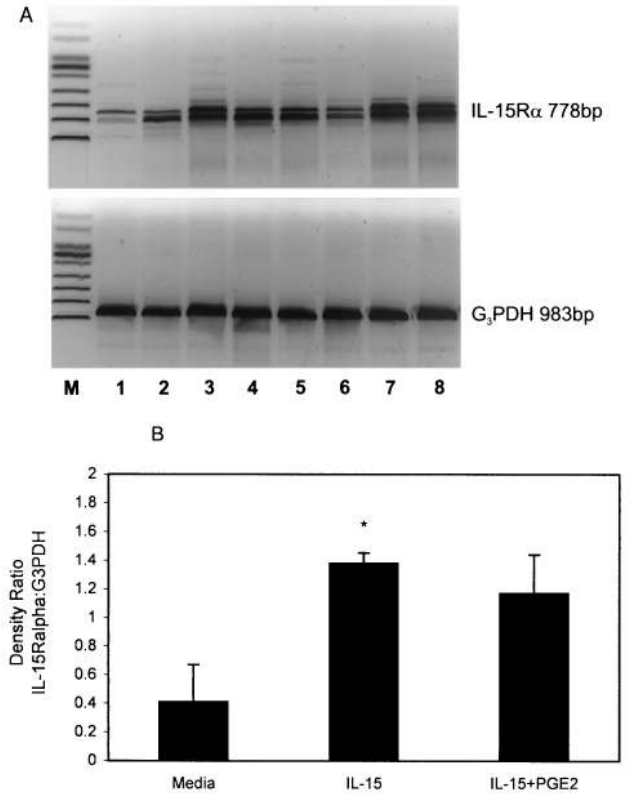

FIGURE 3. Expression of IL-15Rα on human NK cells by RT-PCR. *A*, Human NK cells expressed constitutive levels of IL-15Rα that was increased by IL-15 in a dose-dependent manner. *Lane M,* m.w. marker; *lane 1,* control; *lane 2,* 1 ng/ml IL-15 at 2 h; *lane 3,* 10 ng/ml IL-15 at 2 h; *lane 4,* 100 ng/ml IL-15 at 2 h; *lane 5,* 200 ng/ml IL-15 at 2 h; *lane 6,* 200 ng/ml IL-15 plus anti-IL-15 (5×) at 2 h; *lane 7,* 200 ng/ml IL-15 at 8 h; *lane 8,* 200 ng/ml IL-15 at 18 h. Data shown here is representative of at least three separate experiments. PGE_2 did not significantly suppress up-regulation of IL-15Rα at any time. *B,* The density ratio of IL-15Rα/G3PDH at 18 h. *, Significant at <0.05 as compared with media.

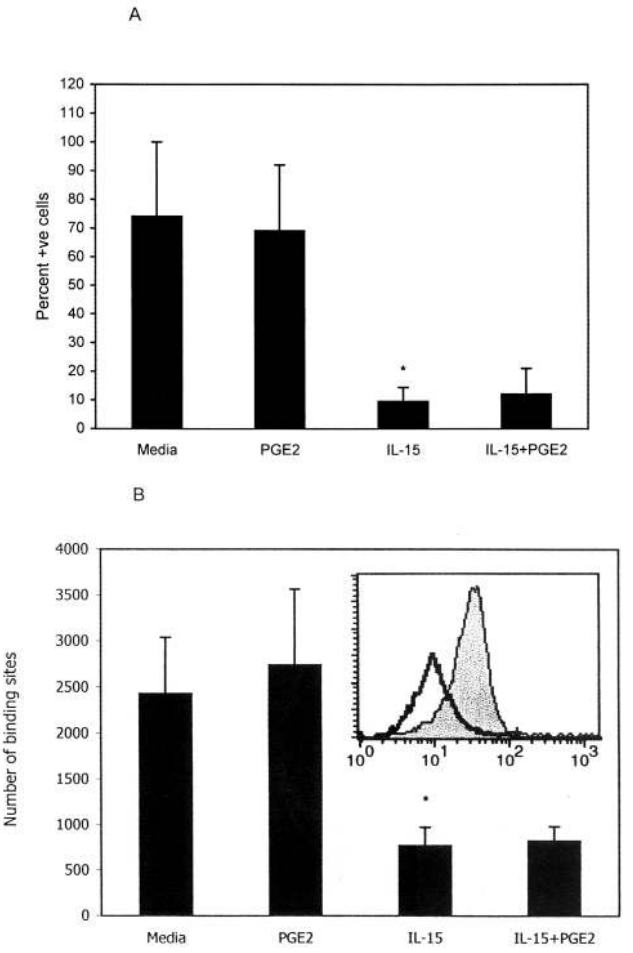

FIGURE 4. Expression of IL-2Rβ on NK cells. Purified human NK cells (2×10^6/ml) were cultured for 2 days with or without 200 ng/ml IL-15 and stained with anti-IL-2Rβ-PE Ab. Cells were analyzed with a flow cytometer. The percent of positive cells (*A*) and number of binding sites (*B*) are shown. Data ($n = 5$) are expressed as mean ± SD. *, Significant at $p < 0.05$ as compared with media. **, Significant at $p < 0.05$ as compared with IL-15. Inset shows histograms of cell counts vs fluorescent intensity for medium (gray) and IL-15 treatments of NK cells. Histograms for PGE_2 and IL-15 plus PGE_2 treatments were comparable to those of medium and IL-15, respectively.

Expression of IL-2Rβ and γ_c-chain on human NK cells and their modulation by IL-15 and PGE_2

After 2 days in culture with or without IL-15 and/or PGE_2, 2×10^6/ml purified human NK cells were analyzed for the expression of IL-2Rβ or γ_c-chain by flow cytometry. High levels of surface IL-2Rβ were observed on human NK cells. Both the percent of positive cells and the number of binding sites for IL-2Rβ were significantly less after IL-15 treatment, probably due to internalization; PGE_2 had no effect (Fig. 4, *A* and *B*). In contrast, the percent of positive cells and the number of binding sites for γ_c-chain were increased after IL-15 treatment (Fig. 5, *A* and *B*). When PGE_2 was added to these cultures, a significant decrease in the percent of positive cells and binding sites was observed. Effects of IL-15 and PGE_2 on the expression of surface IL-2Rβ and γ_c-chain were observed even at days 1 and 3 (Table I).

Transcriptional levels of IL-2Rβ and γ_c-chain were also measured by RT-PCR. IL-15 or IL-15 and PGE_2 cotreatment did not significantly affect constitutive expression of γ_c-chain although a consistent increase in the expression of γ_c-chain was observed after IL-15 treatment (Fig. 6, *lane 5* vs *7*). Similar results were obtained for the expression of IL-2Rβ at the mRNA level (data not shown). Suppression of IL-15-mediated IFN-γ production or γ_c-chain expression was not due to the endogenous production of IL-4, IL-10, or TGF-β_1 by NK cells because neutralizing Abs to these cytokines failed to reverse the suppression (Table II). These suppressive cytokines were not detected in NK cell culture supernatants at any time (data not shown).

Discussion

NK cells can be stimulated by cytokines such as IL-2, IL-12, IL-15, and IL-18. Upon stimulation, NK cells up-regulate cytolytic capacity and secrete higher levels of IFN-γ and other cytokines. Because NK activity is suppressed in cancer-bearing hosts, cytokine treatments may offer one approach to restore or boost NK functions. However, clinical trials for cytokine immunotherapy of cancer have had limited success so far. This failure could be due to the fact that high amounts of suppressive factors are present in tumor-microenvironment. A better understanding of functional interactions between tumor-derived suppressive mediators and cytokine-activated NK cells is essential for designing strategies for cytokine based immunotherapy in cancer patients. Along these lines it is known that tumor-derived PGE_2 is one such suppressive mediator that inhibits host immunity and inhibition of PGE_2 production has been shown to increase anti-tumor response (18). We had recently reported that PGE_2 could be suppressive if present

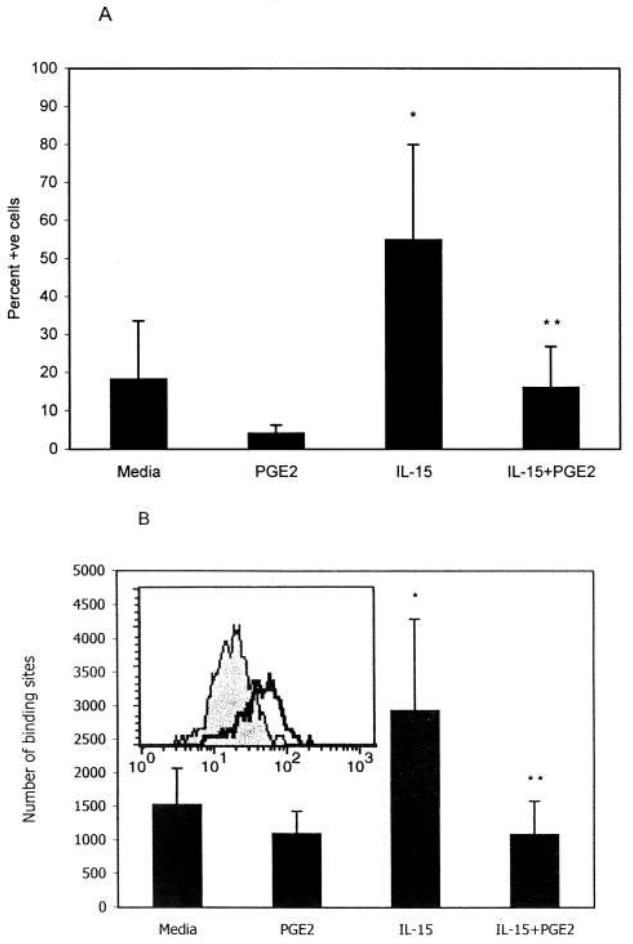

FIGURE 5. Expression of γ_c-chain on NK cells. Purified human NK cells (2×10^6/ml) were cultured for 2 days with or without 200 ng/ml IL-15 and stained with anti-IL-2Rγ-PE Ab. Cells were analyzed by flow cytometer. The percent of positive cells (*A*) and number of binding sites (*B*) are shown. Data ($n = 5$) are expressed as mean ± SD. *, Significant at $p < 0.05$ as compared with media. **, Significant at $p < 0.05$ as compared with IL-15. Inset shows histograms of cell counts vs fluorescent intensity for medium (gray) and IL-15 treatments of NK cells. Histograms for PGE_2, and IL-15 plus PGE_2 treatments were comparable to that of medium.

simultaneously with IL-15 in the NK cell cultures (19). Our results clearly showed that PGE_2 suppressed IL-15-mediated human NK cell function. Both cytotoxicity and IFN-γ production were suppressed in a dose-dependent manner. PGE_2 is known to inhibit Th_1 cytokine responses and enhance Th_2 responses (20, 21). It has also been reported that the presence of PGE_2 before or during T cell activation is necessary to exert its inhibitory effect (21). Because large amounts of PGE_2 are present near tumors, it is important to

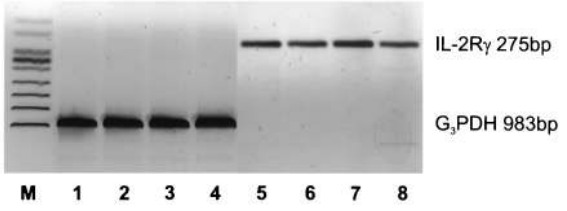

FIGURE 6. Expression of γ_c-chain mRNA (*lanes 5–8*). NK cells were cultured for 18 h with media (*lanes 1* and *5*), 200 ng/ml PGE_2 (*lanes 2* and *6*), 200ng/ml IL-15 (*lanes 3* and *7*), and IL-15 plus PGE_2 (*lanes 4* and *8*).

Table II. *Suppression of INF-γ secretion and surface γ_c-chain expression by PGE_2 was not due to the endogenous production of IL-4, IL-10, or TGF-β_1*[a]

Treatment	INF-γ (pg/ml)	Surface γ_c-Chain Expression (% positive cells)
Medium	ND	8
IL-15	38,693	22
IL-15 + PGE_2	3,996	0
IL-15 + PGE_2 + anti-IL-4	ND	0
IL-15 + PGE_2 + anti-IL-10	ND	0
IL-15 + PGE_2 + anti-TGF-β_1	ND	0

[a] The detection limit of INF-γ ELISA was 25 pg/ml. NK cells (2×10^6/ml) were cultured for 2 days with or without 200 ng/ml IL-15, 200 ng/ml PGE_2, 10 μg/ml anti-IL-4, 10 μg/ml anti-IL-10, or 10 μg/ml anti-TGF-β_1. Data from a representative experiment (one of three) are shown.

evaluate NK cell function in the presence of not only cytokines but also PGE_2. Our data showed that PGE_2 significantly suppressed IFN-γ production by IL-15-activated NK cells. IFN-γ production at the transcriptional level was not inhibited at 2 h. However, by 18 h complete inhibition was observed. Most studies have reported suppression of IFN-γ at the secretional level. Our studies demonstrate that the suppression is also at the mRNA level. The concentration of PGE_2 (200ng/ml) at which strong suppressive effects were seen is comparable with those reported in the literature and is biologically relevant.

There could be several mechanisms by which PGE_2 could have suppressed IL-15-mediated NK function. IL-15 binds to an IL-15R complex, which is composed of a specific subunit IL-15Rα as well as IL-2Rβ and γ_c-chain (22, 23). It is possible that PGE_2 knocks out one or more of these receptor chains. Recently one study reported that PGE_2 inhibited responsiveness of activated human PBMCs and T cells through the suppression of expression of IL-12Rβ_1 (24). PGE_2 is also known to increase IL-1R (25), downregulate IL-2 (26), and TGFβR (27), and release soluble receptors for TNF-α (28). However, to our knowledge, no information is available on how PGE_2 may regulate IL-15R on NK cells. Our results showed a strong constitutive expression of IL-15Rα at the mRNA level but very little surface expression was observed by flow cytometric analysis. IL-15 treatment increased IL-15Rα mRNA expression in a dose-dependent manner and it was inhibited by anti-IL-15 Ab. This increase in IL-15Rα mRNA expression was seen as early as 2 h, reached a peak at 8 h, and was still strongly present at 18 h. PGE_2 treatment did not significantly suppress this expression. This is in contrast with the studies that have reported inhibition of cell surface expression of IL-2Rα and IL-2Rα-specific mRNA after PGE_2 treatment in T lymphocytes (26). These differences may be due to the cell type (NK vs T) or to differential regulation of different cytokine receptors by PGE_2. Recently, it was reported that the role of IL-15Rα in the high affinity IL-15R complex was distinct from the IL-2Rα in the high affinity IL-2R complex (29).

The common γ_c-chain is shared by IL-2, IL-4, IL-7, IL-9, and IL-15R. It is expressed at low levels on the surface of T lymphocytes (30, 31) and on monocytes (32). Our results clearly showed that the low levels of constitutive surface expression of γ_c-chain on NK cells were significantly increased after IL-15 stimulation. The constitutive expression of γ_c-chain mRNA was higher, but not significant, after IL-15 treatment; PGE_2 had no effect on this expression. We have also observed an intracellular expression of γ_c-chain by flow cytometric analysis that was decreased after

IL-15 stimulation and this decrease was partially reversed after PGE_2 treatment (data not shown). As reported in recent studies (33), it appears that γ_c-chain subunit is stored inside cells and is translocated to the surface after activation. This suggests that after IL-15 activation of human NK cells, intracellularly stored γ_c-chain was translocated to the surface; PGE_2 may have modulated this translocation resulting in down-regulation of surface γ_c-chain subunit. Studies have shown that T cells express TCRβ-chains intracellularly, which are transported to the surface after TCRα-chain is synthesized. Although this and some other similar models are reported (34, 35), expression of IL-2Rβ and γ_c-chain on human NK cells is unique especially after IL-15 activation. The surface expression of IL-2Rβ was consistently decreased after IL-15 stimulation whereas expression of γ_c-chain was significantly increased at the same time. It is possible that there may be some connection in their expression pattern. Down-regulation of IL-2Rβ is probably due to internalization of IL-15/IL-2Rβ complex. Because expression of γ_c-chain was increased, it is tempting to speculate that these two events are interrelated and internalization of IL-15/IL-2Rβ complex helped translocation of γ_c-chain subunit to the surface. However, this may not be the mechanism because PGE_2 treatment did not affect expression of IL-2Rβ but γ_c-chain expression was significantly decreased.

The suppressive cytokines IL-4, IL-10, and TGF-β_1 were not detected in the supernatants. In addition, to rule out the suppressive effects of endogenous production of undetectable levels of these cytokines, neutralizing Abs for IL-4, IL-10, or TGF-β_1 were added to the cultures; however, they did not reverse the suppression (Table II). Therefore, the suppressive effect of PGE_2 was not due to these cytokines. There are conflicting reports regarding the effect of IL-13 on NK cells. It has been reported previously that the functional IL-13R is not expressed on NK cells (36) and lymphokine-activated killer (37) cells. Some recent studies have shown that although IL-13 suppressed IFN-γ production in T cell cultures, in primary human NK cell cultures IL-13 induced low levels of IFN-γ production (38). Therefore, we did not consider IL-13 as a candidate for the suppressive effect of PGE_2. It cannot be ruled out at this time that some unknown cytokine secreted in these cultures could have suppressed IL-15-stimulated NK cell function.

Recently, many therapeutic approaches are focused on cytokine receptor subunits and signaling pathways. Clinical trials with Mikβ_1 Ab directed toward IL-2/15Rβ are being conducted. Other therapeutic efforts include development of an inhibitor of Janus kinase (JAK)3 signaling molecule that is used by IL-2, IL-4, IL-7, IL-9, and IL-15. Because these cytokines share receptors and signaling pathways such as JAK/STAT, more information about their receptor expression patterns and precise roles after activation by cytokines will be useful. It is possible that different cytokines may use different receptor subunits for internalization and, therefore, an Ab directed toward a particular receptor subunit may differentially block the stimulation by cytokines that use a common receptor subunit. In summary, results of this study revealed regulation of IL-15R expression by IL-15 and PGE_2 in human NK cells. In particular, our data on down-regulation of γ_c-chain on human NK cells by PGE_2 is interesting because the importance of γ_c-chain in immune activation is known. For example, mutation of γ_c-chain protein in mice and humans results in X-linked severe combined immunodeficiency, which is characterized by impaired development of T cells, B cells, and NK cells (39). Furthermore, a defect in γ_c-chain expression leads to impaired activation of JAK3/STAT5 pathway. Studies are in progress to investigate signal transduction molecules that may be affected by PGE_2.

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