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IL-1 and Tumor Necrosis Factor-α Each Up-Regulate Both the Expression of IFN-γ Receptors and Enhance IFN-γ-Induced HLA-DR Expression on Human Monocytes and a Human Monocytic Cell Line (THP-1)

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Abstract. Stimulation of human blood monocytes (adherent mononuclear cells) and the monocytic cell line, THP-1, by IL-1 or TNF-α leads to the up-regulation of IFN-γ receptors. Scatchard analysis using ^{125}I-IFN-γ revealed a twofold increase in the number of IFN-γ receptors on THP-1 cells without an alteration in the affinity of the receptor. The potential functional significance of this induction of IFN-γ receptors on monocytes and THP-1 cells was investigated by examining the effect of IFN-γ on MHC class II Ag expression by these cells. Both IL-1 and TNF-α enhanced the IFN-γ-induced HLA-DR expression (>twofold) and this effect was inhibited by antibody to IFN-γ. In the case of human monocytes, IL-1 or TNF-α, each by themselves also increased HLA-DR expression, which was also abrogated by antibody to IFN-γ. The data suggest that the immunopotentiating effects of IL-1 and TNF-α are mediated in part by enhancing IFN-γ receptor expression on monocytes and macrophages. This presumably would increase the capacity of IFN-γ to activate macrophages, enabling them to express HLA-DR and present Ag more effectively. *Journal of Immunology,* 1993, 150: 1205.

IFN-γ, a lymphokine produced by activated T cells, is a potent activator of monocytes and macrophages. IFN-γ initiates its biologic effects by binding to specific cell-surface receptors on these cells (1, 2). TNF-α is known to synergize with IFN-γ, resulting in a number of augmented biologic effects, such as antimicrobial and antitumor activity (3, 4). A number of cytokines, including IL-1 and TNF-α, are known to regulate the expression of receptors for other cytokines and, thereby, amplify their biologic responses both in vitro and in vivo (5–12). It is of interest to study the regulation of IFN-γ receptors by cytokines that synergize with IFN-γ. Therefore, we investigated whether IL-1 and TNF-α could affect the binding of IFN-γ to its receptors and whether this influences functional effects of IFN-γ, such as its capacity to induce the expression of MHC class II Ag on monocytes and the monocytic cell line, THP-1.

Materials and Methods

Materials

Human rIL-1α (sp. act. 2×10^7 U/mg), and human rIL-1β (sp. act. 9×10^5 U/mg) were provided by Dainippon Pharmaceutical Co. Inc. (Osaka, Japan) and Du Pont (Glenolden, PA), respectively. Human rTNF-α (sp. act. 2×10^7 U/mg) and peroxidase-conjugated goat anti-mouse

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IgG antibody were purchased from Boehringer-Mannheim (Indianapolis, IN). Antiserum against human IFN-γ (1000 neutralizing U/ml) was obtained from Interferon Sciences (New Brunswick, NJ). The specificity of this antibody was documented by its ability to block the antiviral activity of IFN-γ but not that of IFN-α or IFN-β in WISH cells. This antibody was also effective in blocking the HLA-DR enhancement induced by IFN-γ, but not that by IL-4, in PBMC. Anti-human HLA-DR and purified mouse IgG were from Becton Dickinson (Mountain View, CA). OKM1 and Leu M1 (anti-human monocyte/macrophage) antibodies were kindly provided by Dr. John Ortaldo, LEI, BRMP, National Cancer Institutes, Frederick, MD.

Cell culture

The human monocytic leukemia cell line, THP-1, was cultured in RPMI 1640 and 10% FCS at 1×10^6 cells/ml with or without cytokines. Tubes containing 2 ml of cells were used for ^{125}I-IFN-γ binding and 96-well plates with 0.1 ml volume of cell suspension per well were used for HLA-DR determination.

Human PBMC were isolated by Ficoll-Hypaque density centrifugation of heparinized blood from normal donors. PBMNC were plated at 0.8 to 1.5×10^7 cells/well in 24-well plates (for ^{125}I-IFN-γ binding assay) or at 0.8 to 1.5×10^6 cells/well in 96-well plates (for HLA-DR assay) in RPMI containing 10% heat-inactivated FCS and allowed to adhere for 2 h at 37°C. Nonadherent cells were removed by four vigorous washings of the cultures. The remaining adherent PBMC were 95 to 99% esterase-positive cells. Cells were then incubated with cytokines for 16 to 19 h before assays of ^{125}I-IFN-γ binding or for 1 to 2 days before assays of HLA-DR expression.

IFN-γ receptor binding

Binding studies with ^{125}I-IFN-γ (sp. act. 675 Ci/mmol, Amersham, Corp., Arlington Heights, IL) were performed at 4°C for 80 min in RPMI medium containing 10% heat-inactivated FCS, 0.1% NaN_3, and 20 mM HEPES. For Scatchard analysis, THP-1 cells were incubated with seven serial twofold dilutions of ^{125}I-IFN-γ, ranging from 0.1 to 2 nM in the absence or presence of 250-fold molar excess of unlabeled human rIFN-γ (Collaborative Research, Boston, MA) to determine total and nonspecific binding, respectively. The binding reaction mixture (0.2 ml) was then overlaid on a 20% sucrose gradient and centrifuged ($10{,}000 \times g$) for 2 min. Supernatant containing free ligand was removed and the cpm of ^{125}I-IFN-γ bound to cells was determined by excising tips of tubes and counting them in a gamma-counter (Beckman Instruments Inc., Irvine, CA). Nonspecific binding usually did not exceed 25% of the total binding.

Binding of ^{125}I-IFN-γ to human adherent PBMC was determined directly on 24-well plates. Cells were washed after cytokine treatment with PBS, and 0.3 ml of binding buffer containing 0.8 nM of ^{125}I-IFN-γ was added. After binding at 4°C for 80 min, supernatants were removed and plates were washed twice with cold PBS containing 0.1% NaN_3. Cells were solubilized with 0.4 ml of 0.2 N NaOH and the radioactivity was determined in a gamma-counter. Nonspecific binding was determined as for THP-1 cells by using a 250-fold molar excess of unlabeled human rIFN-γ, and was subtracted from total binding to calculate specific binding. To calculate the number of receptors and K_d, specific binding data were plotted according to Scatchard (13). A least squares linear regression was used to give an objective measurement of goodness of fit in calculating the K_d.

Measurement of HLA-DR expression on cells by ELISA

Cells in 96-well plates were washed with PBS and fixed with ethanol. Plates were incubated with 50 μl/well of PBS containing 100 μg/ml human IgG (Sigma Chemical Co., St. Louis, MO), 10% heat-inactivated FCS, 0.05% Tween 20, 5 μl of mouse mAb (anti-HLA-DR, or OKM1 or Leu M1) at 37°C for 50 min, and were washed twice with buffer (PBS with 2% heat-inactivated FCS and 0.05% Tween 20). 0.1 ml of horseradish peroxidase-conjugated anti-mouse antibody was added to each well and incubated at 37°C for 30 min. Plates were washed three times and the bound enzyme was quantitated at 410 nm (MR600 Microplate Reader, Dynatech, Alexandria, VA) by using 2–2'-azino-di-(3-ethylbenzthiazoline sulfonate) and hydrogen peroxide as substrates (Kirkegaard and Perry Lab, Gaithersburg, MD). Controls of another surface Ag (Leu M1 and OKM1 for THP-1 and monocytes, respectively) not modulated by cytokine treatments were included in each experiment.

Results

IL-1 and TNF-α enhance binding of ^{125}I-IFN-γ to THP-1 cells and adherent PBMC

Expression of IFN-γ receptors on THP-1 cells was assessed by the binding of ^{125}I-labeled IFN-γ. Table I shows the effect of varying doses of IL-1 and TNF-α on the expression of IFN-γ receptors on these cells. There was little variation in the induction of these receptors over a wide dose range (10–200 ng/ml) of IL-1. However, TNF-α at lower concentrations (200 U/ml) was not effective. Thus, after 19 h exposure of these cells to 100 ng/ml IL-1, the binding to IFN-γ receptors increased to 146% of the untreated, medium control (Table I). TNF-α at 2000 U/ml similarly increased the binding of IFN-γ to its receptors by 147%.

The time course for the enhancement of IFN-γ receptors

Table I
Effect of varying doses of IL-1 and TNF-α on ^{125}I-IFN-γ binding on THP-1 cells

Addition[a]	Specific Binding[b]	
	cpm	% Bound
Medium	12700 ± 800	100
IL-1, 10 ng/ml	18200 ± 1200	143
100 ng/ml	19000 ± 1100	150
200 ng/ml	18600 ± 900	146
TNF-α, 200 U/ml	14100 ± 900	111
2000 U/ml	18700 ± 800	147

[a] THP-1 cells (2×10^6) were treated at 37°C for 19 h before ^{125}I-IFN-γ binding.
[b] Average ± SD for duplicates from one of two experiments.

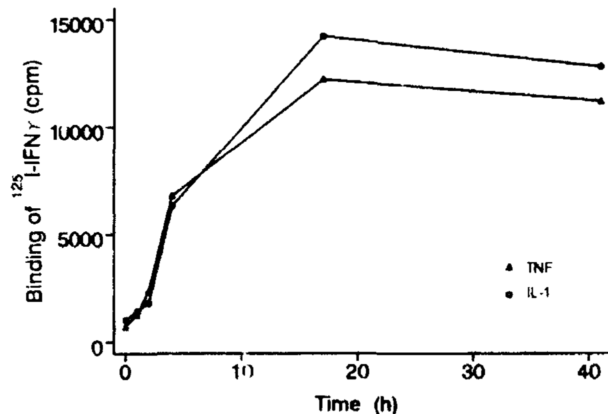

FIGURE 1. Time course of ^{125}I-IFNγ binding to IL-1 and TNF-α-treated THP-1 cells. THP-1 cells (2×10^6) were incubated with 100 ng/ml of IL-1 (○) or 2000 U/ml of TNF-α (△) for various time at 37°C. ^{125}I-IFN-γ binding was determined with 0.8 nM of ^{125}I-IFN-γ as described in *Materials and Methods.* Untreated cells bound 14,000 cpm of ^{125}I-IFN-γ. This quantity was subtracted from the bound cpm shown.

Table II
Up-regulation of IFN-γ receptors by IL-1 and TNF-α on human peripheral blood monocytes

Addition[a]	Specific Binding[b]	
	cpm	% Bound
Medium	15400 ± 900	100
IL-1, 100 ng/ml	21700 ± 1300	141
TNF-α, 2000 U/ml	22300 ± 1700	144

[a] Adherent PBMC (1.2×10^6) were treated at 37°C for 19 h before ^{125}I-IFN-γ binding.
[b] Average ± SD for duplicates from one of three experiments.

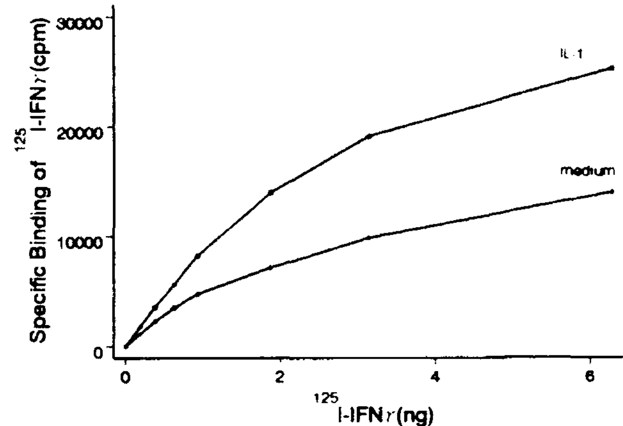

FIGURE 2. Binding of ^{125}I-IFN-γ to untreated and IL-1 treated THP-1 cells. THP-1 cells were incubated with 50 ng/ml of IL-1 (○) or medium (◇) for 16 h at 37°C. ^{125}I-IFN-γ binding was determined as described in *Materials and Methods.*

by IL-1 and TNF-α was also examined. Significant enhancement became detectable at 4 h in IL-1 or TNF-α-treated THP-1 cells (Fig. 1). Enhancement of IFN-γ receptors was maximal at 17 h.

We next studied the effect of these two monokines on the expression of IFN-γ receptors on adherent PBMC from normal adult volunteers. IL-1 and TNF-α each increased the binding of ^{125}I-IFN-γ to human PBMC (Table II). Thus, the amount of bound ^{125}I-IFN-γ was increased to 141 and 144% by IL-1 and TNF-α, respectively.

Increase of IFN-γ receptor number on THP-1 cells

Stimulation of ^{125}I-IFN-γ binding to adherent PBMC and THP-1 cells may be due to increased receptor affinity or increased receptor number. To address this issue, THP-1 cells were incubated in the absence or presence of IL-1 at 37°C for 16 h and binding was assayed (Fig. 2). Scatchard analysis of the binding data revealed that unstimulated THP-1 cells displayed 3200 receptors per cell with a K_d of 1.4×10^{-10} M, whereas 6900 receptors per cell with a K_d of 1.5×10^{-10} M were found on IL-1-treated cells (Fig. 3). Thus, the increase in IFN-γ binding capacity upon IL-1 treatment on THP-1 cells was caused by an increase in the receptor number and was not due to an alteration in the affinity of binding. Due to the high doses of TNF-α used, we did not assess the affinity of binding in TNF-α-treated cells by Scatchard analyses.

Enhancement of IFN-γ-induced HLA-DR expression on THP-1 cells by IL-1 and TNF-α

These findings suggested that IL-1 and TNF-α might facilitate the effects of IFN-γ. We and others previously reported that IFN-γ induces monocyte HLA-DR Ag expression (14–16). We therefore investigated whether the increase in IFN-γ receptors would lead to an increase in HLA-DR expression on these cells.

The basal level of HLA-DR expression was low on THP-1 cells (Fig. 4). IL-1 or TNF-α alone did not induce HLA-DR expression, whereas IFN-γ induced HLA-DR in a dose-dependent manner. IL-1 enhanced the IFN-γ induction of HLA-DR on THP-1 cells by up to twofold at various concentrations tested. Similar results were obtained with TNF-α and IFN-γ; HLA-DR expression was

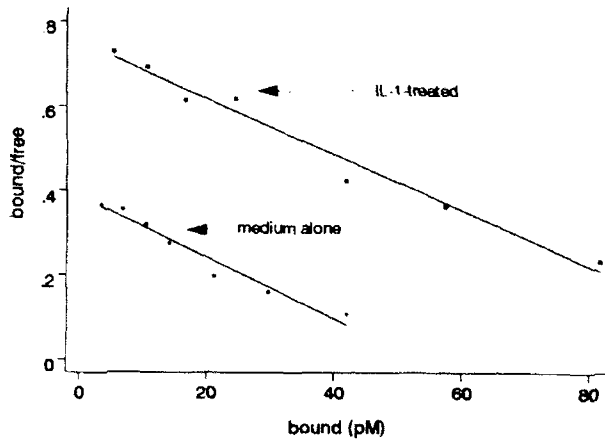

FIGURE 3. Scatchard analysis of ^{125}I-IFN-γ binding to untreated and IL-1-treated THP-1 cells. Data were taken from Figure 2 and replotted according to Scatchard. Shown is one representative experiment of two.

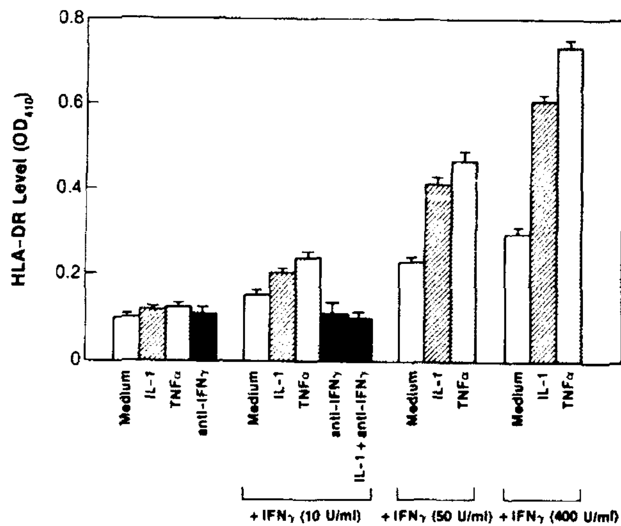

FIGURE 4. Enhancement of IFN-γ-induced HLA-DR expression on THP-1 cells by IL-1 and TNF-α. THP-1 cells (1 × 10^5) were cultured in the presence of IL-1 (100 ng/ml), TNF-α (2000 U/ml), IFN-γ (U/ml as indicated), anti-IFN-γ (50 neutralizing unit/ml) or medium alone for 48 h at 37°C. Cells were quantitated for HLA-DR expression by ELISA. Data were normalized to the surface expression of an unmodulated antigen by using Leu M1 antibody (OD_{410} = 0.42). *Bar* represents the mean and the SD of duplicates. Results were representative of three experiments.

progressively increased with greater doses of IFN-γ used in these experiments (10 to 400 U/ml). The enhancement of HLA-DR expression by cytokines was maximal at 48 h (data not shown). Leu M1, a marker for human myelomonocytic cells, was used as a control, and its expression was not altered by these cytokine treatments. Antiserum to IFN-γ blocked both the IL-1 and IFN-γ as well as TNF-α- and IFN-γ-induced HLA-DR expression completely.

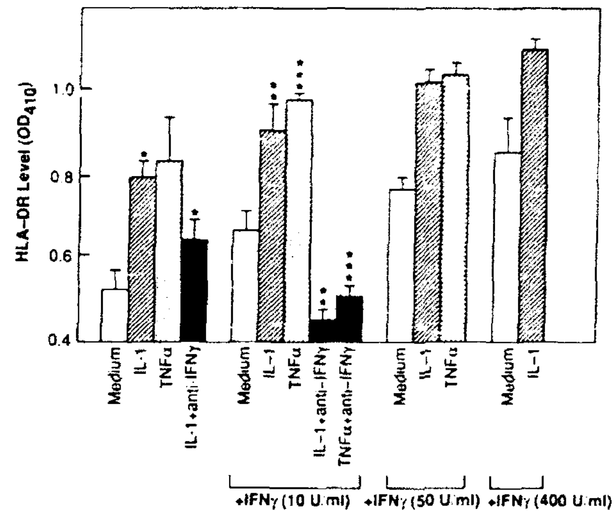

FIGURE 5. Enhancement of HLA-DR expression on human monocytes by IL-1, TNF-α, and IFN-γ. Adherent PBMC (1 × 10^5) were cultured in the presence of IL-1 (100 ng/ml), TNF-α (2,000 U/ml), IFN-γ (U/ml as indicated), anti-IFN-γ (50 NU/ml), or medium alone for 48 h at 37°C. Cells were quantitated for HLA-DR expression by ELISA. Data were normalized to the surface expression of an unmodulated Ag by using OKM1 antibody (OD_{410} = 0.72). Differences in HLA-DR expression were analyzed for significance by Student's *t*-test for paired samples (*p = 0.06, **p = 0.01, ***p = 0.01). Results were representative of three experiments.

The enhancement of IFN-γ induced HLA-DR expression on THP-1 cells by IL-1 or TNF-α was seen only if cells were treated first with IL-1 or TNF-α and then with IFN-γ, or simultaneously with IL-1 and IFN-γ or TNF-α and IFN-γ (data not shown). When the treatment with IFN-γ preceded that with IL-1 or TNF-α, HLA-DR induction could be accounted for by the action of IFN-γ alone. Thus, there was a temporal correlation between IL-1- or TNF-α-induced increases in IFN-γ binding and the subsequent HLA-DR enhancement.

Enhancement of HLA-DR expression on adherent PBMNC by IL-1, TNF-α and IFN-γ

We further examined the relationships of these cytokines to the induction of MHC class II Ag on PBMC from normal adult volunteers. Consistent with our previous observation, the constitutive level of HLA-DR Ag on PBMC was quite high (OD = 0.54) (Fig. 5). IFN-γ further raised this level at the various concentrations tested. Despite previous reports that IL-1 does not directly induce class II MHC on macrophages (17, 18), we observed that IL-1 alone significantly enhanced HLA-DR expression on adherent PBMC above the basal level (OD = 0.812). TNF-α also induced HLA-DR expression by itself. Furthermore, as with the THP-1 cells, both IL-1 and

TNF-α potentiated the IFN-γ-induced HLA-DR expression at different doses.

We also investigated whether the induction of MHC class II Ag on adherent PBMC was mediated by IFN-γ. This possibility was supported by the complete inhibition of HLA-DR expression by anti-IFN-γ antibodies when IL-1 and IFN-γ or TNF-α and IFN-γ were used (Fig. 5). Anti-IFN-γ also abrogated the IL-1-mediated induction of HLA-DR expression on these cells. Collectively, these data suggest that increased induction of IFN-γ receptors by IL-1 and TNF-α leads to an enhancement of MHC class II expression on monocytes and THP-1 cells.

Discussion

IL-1 was initially described by immunologists as a macrophage-derived cytokine with comitogenic effects on thymocytes and T lymphocytes (19). In the intervening years, IL-1 has been found to be produced by many other cell types and to have pleiotropic activities (20). Ironically, the paradigm that IL-1 participates in promoting the activation of T lymphocytes by APC has been challenged by recent experiments showing that neither anti-IL-1 receptor antibodies to the type I IL-1R nor IL-1 receptor antagonist has the capacity to interfere with APC-mediated activation of in vitro lymphoproliferation responses (21, 22). Nevertheless, in vivo administration of IL-1 has considerable adjuvant effects and can enhance cellular (23) as well as humoral immune responses (24, 25). The basis for the immunopotentiating effects of IL-1 is therefore now less clear than ever. IL-1 is still believed to promote the production of IL-2 (26) and the expression of the IL-2Rα chain by T and B lymphocytes (27). IL-1 may also augment immune responses by inducing other immunostimulant cytokines such as IL-4 (28), IL-6 (29), and TNF-α (30).

TNF-α was initially discovered as a serum antitumor activity (31) and only recently found to have many immunopotentiating activities that overlap with those of IL-1, perhaps mediated by induction of IL-2Rα (32). TNF-α also is a comitogen for thymocytes (33), but unlike IL-1, TNF-α has previously been reported to induce IFN-γ production (32) and to costimulate with IFN-γ MHC class II expression (34, 35). These capabilities may contribute to the immunostimulant effects of TNF-α in promoting tumor immunity (36) and enhancing antibody production in vitro and in vivo (37, 38).

Our experiments show for the first time that both IL-1 and TNF-α have the capacity to increase the number of binding sites for IFN-γ up to twofold on cultured, adherent human mononuclear cells, and on homogeneous THP-1 cells. The effect of this increase in IFN-γ binding on the as yet unidentified signal transducing peptide for IFN-γ, which is coded for on chromosome 21 (39), remains to be established. However, we were able to show up to twofold increases in MHC class II Ag expression on IL-1 plus IFN-γ as well as on TNF-α plus IFN-γ-stimulated mononuclear and THP-1 cells. Although this merely represents a correlation, nevertheless the relationship between the up-regulation in IFN-γ binding and class II expression was reinforced by the observation that the latter change could be completely blocked by antibody to IFN-γ. The antibody to IFN-γ even blocked the increase in class II expression by mononuclear cells, which was induced by IL-1 or TNF-α by themselves, presumably because some of the contaminant T cells produced low levels of the IFN-γ costimulant. This view is supported by the inability of either IL-1 or TNF-α to induce MHC class II receptors on "pure" THP-1 cells, despite the up-regulation of the IFN-γ binding.

These findings are consistent with a report of a direct correlation of the degree of response to IFN-γ and the number of IFN-γ receptors on different tumor cells (40): cells with higher number of IFN-γ receptors responded to lower concentrations of IFN-γ. Other reports on the magnitudes of induced cytokine receptor levels and their biologic responses vary. For example, TNF-α increased the high-affinity IL-2 receptors on T cells by 50% and enhanced the proliferative response by 30% (6). Up to twofold (5, 7, 8, 12) and occasionally higher levels (9–11) of receptor induction have also been observed. We do not know whether IL-1 or TNF-α is involved in augmenting signal amplification pathways subsequent to receptor binding.

The magnitude of the increase of IFN-γ receptors by IL-1 or TNF-α on adherent PBMC or THP-1 cells agrees with reports of other cytokine-induced receptor levels (5–8, 12). A number of other cytokines including IL-4, IL-6, and IL-10 did not induce IFN-γ receptors (data not shown). Thus, this activity was unique for IL-1 and TNF-α. Although the constitutive level of HLA-DR was high in adult adherent PBMC, both IL-1 and TNF-α were capable of increasing this basal level further. The combination of cytokines, either IL-1 and IFN-γ or TNF-α and IFN-γ, enhanced the HLA-DR expression even further. This enhanced HLA-DR expression is important for biologic function because it has been shown that T cell proliferative response to Ag is proportional to the number of Ia molecules on the surface of APC (41).

Reports on the mechanism for the increase of IFN-γ receptors by TNF-α were published recently (42, 43). TNF-α-mediated up-regulation of IFN-γ receptors was due to an increase in transcriptional activity of the IFN-γ receptor gene in THP-1 cells (43). Whether IL-1 is similarly upregulating the gene expression for the IFN-γ receptor remains to be ascertained.

The exposure of cells to IL-1 or TNF-α had to antecede or be simultaneous with IFN-γ to obtain enhanced

HLA-DR expression and the increase in IFN-γ receptor induction anteceded increases in HLA-DR expression. Furthermore, the induction of HLA-DR expression was inhibited by cycloheximide (our unpublished observation) suggesting that new protein synthesis is required for the enhancement of HLA-DR expression. More studies are needed to definitively show a causal relationship between the up-regulation of IFN-γ binding and MHC class II expression. However, this pathway does provide an attractive means by which IL-1 as well as TNF-α may exert some of their immunostimulating effects and may contribute to the protective effects of IL-1 and TNF-α and up-regulation of class II MHC by IL-1 and TNF-α recently detected in normal and SCID mice (44, 45).

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