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REGULATION OF MHC CLASS II ANTIGEN EXPRESSION

Opposing Effects of Tumor Necrosis Factor-α on IFN-γ-Induced HLA-DR and Ia Expression Depends on the Maturation and Differentiation Stage of the Cell

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MHC class II induction by cytokines has been suggested to play a major role in the initiation and propagation of immune and autoimmune processes. TNF-α has been found both to enhance and also to inhibit IFN-γ-induced MHC class II expression. In the present studies, the effect of TNF-α on IFN-γ induced MHC class II expression was tested in various cell lines. On the basis of the data, we propose that, depending on the stage of differentiation and maturation of the cells, TNF-α might synergize or antagonize the affects of IFN-γ on the regulation of MHC class II expression. Thus, in immature cells such as HL-60 or THP-1, TNF-α enhances IFN-γ-induced class II expression. However, when differentiation was induced in these cells by TPA or IFN-γ, the additive effect of TNF-α on the IFN-γ induced DR expression was eliminated. Furthermore, TNF-α down-regulates the IFN-γ-induced class II expression in differentiated cells such as human skin fibroblasts or activated macrophages. In bone marrow cells induced to differentiate in vitro, TNF-α decreased the IFN-γ-induced MHC class II expression in a maturation-dependent fashion. These results provide a rational explanation for the conflicting reports regarding the effect of TNF-α on IFN-γ-induced class II expression. But more importantly they may be relevant to the biologic function of TNF-α. Thus, we show that TNF-α-treated mice have reduced level of Ia expression on peritoneal macrophages and in vivo treatment with TNF-α antagonizes the ability of IFN-γ to induce class II expression on these macrophages.

Class II MHC molecules play a major role in the initiation of immune responses (1, 2). Important functions include presentation of soluble Ag to helper T lymphocytes and stimulation of alloreactivity. The induction of an immune response to protein Ag depends on the recognition by T cells of antigenic determinants in association with MHC class II molecules (termed DR, DQ, and DP in man). The normal expression of class II MHC genes show a limited tissue distribution, primarily to activated macrophages, B lymphocytes, dendritic cells, and activated T cells. Aberrant expression of class II MHC molecules has been noted in autoimmune diseases (3, 4) and it has been demonstrated that this may lead to chronic stimulation of autoreactive T cells (5).

The amount of class II MHC Ag expressed on the cell surface appears to be an important regulatory mechanism of APC function (6). This aspect of APC function has both qualitative and quantitative characteristics. It has been shown (7–10) that the absolute level of class II MHC expression on APC contributes in a quantitative fashion to the efficiency of T cell activation. Given the promiscuous nature of MHC-Ag binding with relatively low affinity and specificity for peptide Ag (11, 12), the dependency of the efficiency of Ag presentation on the density of class II molecules on the cell surface may be actually of more importance than previously estimated.

IFN-γ has been shown to increase the expression of class II genes in a variety of human and murine cell types (13–17). IFN-γ is produced exclusively by cells of the T lymphocytic lineage, thus class II expression appears to be tightly regulated, its level being dependent on a product of T cells capable of recognizing and responding to Ag. IFN-γ is part of a network of interactive lympokines and monokines that orchestrate immunologic events. In this regard, it has been shown that TNF-α has both similar or additive effects to IFN-γ in various in vitro models (18, 19). Moreover, it has been postulated that the in vivo effects of TNF-α and IFN-γ are dependent on each other (20, 21). TNF-α, a cytokine produced by activated macrophages and T cells, has been found both to enhance IFN-γ-induced class II expression (22–24), and paradoxically also to inhibit its expression (25, 26).

In the present study, we analyzed the relationship between IFN-γ and TNF-α regarding MHC class II expression in a variety of cells. We show that the differentiation state of the cell may determine its susceptibility to induction of class II MHC proteins by IFN-γ and TNF-α. The results may suggest a rational explanation for the apparently opposing effects of TNF-α on DR and Ia expression and also may be relevant to the in vivo biologic function of this cytokine.

MATERIALS AND METHODS

Cells and cell cultures. Human skin fibroblasts (strain NLFB) were provided by Dr. V. B. Morhenn (Genentech, Inc., South San Francisco, CA). HL-60 and THP-1 cell lines were purchased from American Type Culture Collection (ATCC), Rockville, MD. Human peripheral monocytes were purified from human blood by Percoll

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density gradient centrifugation and adhesion to plastic dishes. Cells were cultured in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin, and 100 ng/ml streptomycin at 37°C in 5% CO_2. Human peripheral monocytes were cultured in RPMI 1640 supplemented with 5% human A/B serum.

Preparation of mouse bone marrow cells. Bone marrow cells were obtained from the femurs of 5 to 6 wk old C57Bl/6 mice. Marrow plugs were flushed out with PBS, passaged through a 23-gauge needle, washed, and resuspended in complete RPMI medium at a density of 10^6 cells/ml. Macrophages and other adherent cells were removed after incubating the cells for 18 h at 37°C in 5% CO_2 in tissue culture flasks (75 cm^2) as described (27). Non-adherent cells were collected and resuspended in complete medium and plated in 6-well culture dish (Costar, Cambridge, MA) at 2×10^6 cells/well and cultured with M-CSF (25 proliferation units/ml) for 2, 4, or 6 days.

Cytokines and reagents. Human rIFN-γ and recombinant human macrophage CSF were purchased from Genzyme Co. (Boston, MA). Murine rIFN-γ and human and murine rTNF-α were gifts from Genentech, Inc., TPA,[3], a phorbol diester was purchased from Sigma (St. Louis, MO).

Differentiation induction and cytokine treatment. TPA was solubilized in ethanol at a concentration of 10^{-4} M and added to suspension cultures of HL-60 or THP-1 cells at a final concentration of 10^{-7} M. Cells (10^6 cells/ml) were incubated with TPA for 5 days, which converted THP-1 and HL-60 promyelocytic cells into differentiated cells. THP-1 cells and human monocytes were induced to differentiate also by incubation with IFN-γ for 5 days. After differentiation induction, cells were washed and treated with IFN-γ and/or TNF-α at different doses as indicated for 48 h and subsequently analyzed for MHC class II expression by flow cytometry. Under the incubation conditions described there was no loss of cell viability by treatment with any of the cytokines (data not shown).

mRNA dot blot hybridization. Total cytoplasmic RNA was extracted from NLFB human fibroblasts by the method of Chomczynski and Sacchi (28) using the RNAzol (Cinna/Biotex, Friendwood, TX) kit according to the recommendations of the manufacturer. RNA samples were transferred onto Nytran nylon membrane (Schleicher & Schuell, Keene, NH) and hybridized to a HLA-DRα probe. The probe was a 1.3-kb cDNA fragment purchased from ATCC, which was labeled with ^{32}P-dCTP by nick translation. Prehybridization and hybridization of RNA blots were performed as described (29).

To quantify the amount of the specific mRNA, dot-blots were analyzed by scanning with an optical densitometer (Hoefer, San Francisco, CA) equipped with a Macintosh computer. The data are expressed as the ratio of a specific mRNA to β-actin mRNA. Each figure is representative of three or four experiments.

Flow cytometry analysis. After lymphokine stimulation, the cells were washed with PBS and harvested by gentle scraping if they were adherent. Single cell suspensions of mouse macrophages were prepared from peritoneal washouts as described (30). Cells were stained with optimal amounts of mAb as described (30). The following antibodies have been used: anti-I-A^d,MKD6/FITC; anti-I-A^b,24A1/FITC; anti-HLA-DR monomorphic,L243/FITC; anti-MAC-1,M1/70/biotin. Flow microfluorimetry analyses were conducted as described (30). Propiidium iodide (Calbiochem-Behring, La Jolla, CA) was added at a final concentration of 5 μg/ml before analysis to label dead cells. Cells were analyzed on a FACStar plus (FACS II, Becton Dickinson, Mountain View, CA). For each analysis, data from 10000 to 30000 viable cells were collected. Data are representative of at least three experiments.

rTNF-α was conjugated to FITC as described (31). Cells were stained with FITC-TNF-α at a concentration of 850 ng/ml at 4°C for 2 h. Cells were analyzed for TNF-α binding activity by flow microfluorimetry as described (31). Specific binding is defined as the amount of FITC-TNF that can be displaced by 100-fold excess cold TNF.

^{125}I-TNF-receptor binding assay. Binding assay on intact cells in suspension was performed using ^{125}I-TNF-α from Amersham Corp., Arlington Heights, IL following the protocol of Heller et al. (31).

RESULTS

TNF-α down-regulates IFN-γ induced HLA-DR expression in human skin fibroblasts. NLFB human skin fibroblasts were found to be constitutively HLA-DR Ag negative but could be induced to express DR by IFN-γ (Fig. 1). TNF-α alone at different concentrations, ranging from 0.1 to 200 ng/ml failed to induce class II expression on these cells. However, TNF-α (100 ng/ml) reduced the surface expression of HLA-DR induced with 100 U/ml of IFN-γ to control level (Fig. 1*A*) and also decreased significantly the level of HLA-DR expression induced with 400 U/ml of IFN-γ (Fig. 1*B*). This effect of TNF-α could be blocked specifically by antibody to human TNF-α (Fig. 1*B*).

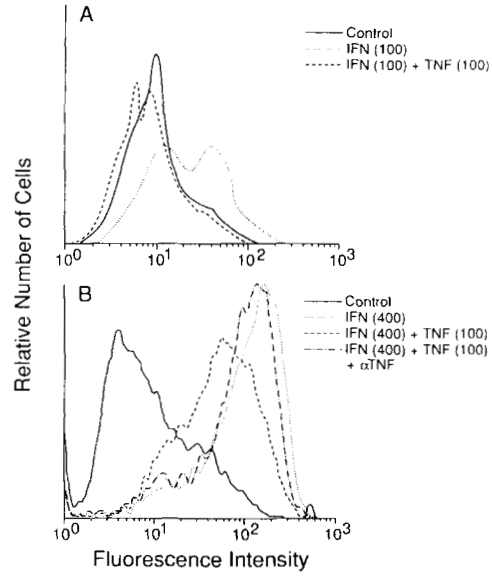

Figure 1. Reduction of IFN-γ induced HLA-DR expression by TNF-α in human skin fibroblasts. Two separate experiments are shown. NLFB cells in *A* were incubated for 48 h in control medium, IFN-γ (100 U/ml) or IFN-γ (100 U/ml) plus TNF-α (100 ng/ml) and stained with FITC-anti-HLA-DR. In *B*, NLFB cells were incubated with IFN-γ (400 U/ml), IFN-γ (400 U/ml) plus TNF-α (100 ng/ml) or IFN-γ (400 U/ml) plus TNF-α (100 ng/ml) plus polyclonal rabbit anti-human TNF-α for 48 h as in *A*.

This antagonistic effect of these two mediators on DR expression seen at the membrane protein level was also observed at the mRNA level. As shown in Figure 2, TNF-α reduced the IFN-γ-induced DR mRNA in a dose-dependent fashion. Ten ng/ml of TNF-α was enough to almost totally block detection of DR mRNA even when IFN-γ was used in concentration of as high as 400 U/ml.

Figure 3 is a time course experiment showing that TNF-α was less effective in antagonizing IFN-γ-induced DR mRNA when added 24 h after IFN-γ, than if added together with or 24 h before IFN-γ. Taken together, these results (Figs. 1 to 3) show that TNF-α down-regulates HLA-DR expression induced by IFN-γ at both mRNA and protein level in fibroblasts.

Induction of differentiation changes effect of TNF-α on HLA-DR expression induced by IFN-γ in monocytic cells. Next, membrane expression of DR Ag in two human monocytic cell lines, HL-60 and THP-1, cultured for 2 days in control medium or medium containing IFN-γ, TNF-α or both were tested. Uninduced cell did not express surface DR molecules but IFN-γ induced a modest increase of DR in THP-1 cells (Fig. 5*A*) and a more significant increase in HL-60 cells (Fig. 4*A*). Addition of TNF-α alone did not induce HLA-DR expression in THP-1 cells (not shown) but definitely enhanced expression of the Ag in HL-60 cells, although to a lesser extent than IFN-γ (Fig. 4*A*). In both cell lines, simultaneous addition of the two mediators resulted in more DR expression than that of each mediator used alone (Figs. 4*A* and 5*A*).

To provide an explanation for the paradoxical results

[3] Abbreviation used in this paper: TPA, 12-O-tetradecanoylphorbol-13-acetate.

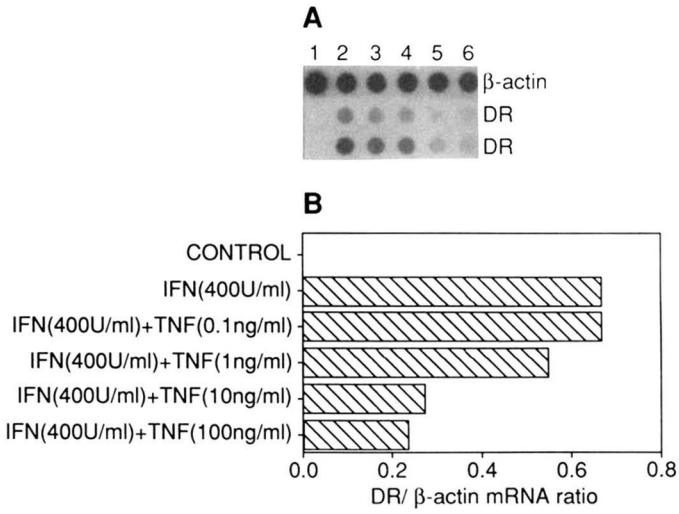

Figure 2. Dose-dependent reduction of the IFN-γ-induced HLA-DR mRNA expression in NLFB cells by TNF-α. Cells were incubated with IFN-γ (400 U/ml) and TNF-α at various concentrations (0.1 to 100 ng/ml) for 72 h. Dot blot hybridization (*A*) was carried out as described in *Materials and Methods*. *Lane 1*, control cells incubated in medium alone, *lane 2*, IFN-γ (400 U/ml); *lane 3*, IFN-γ (400 U/ml) + TNF-α (0.1 ng/ml); *lane 4*, IFN-γ (400 U/ml) + TNF-α (1 ng/ml); *lane 5*, IFN-γ (400 U/ml) + TNF-α (10 ng/ml); *lane 6*, IFN-γ (400 U/ml) + TNF-α (100 ng/ml). To compare quantitatively the levels of specific HLA-DR mRNA levels induced by the different treatments, the amount of RNA loaded in the bottom lanes was three times higher than the upper DR lanes. In *B*, quantification of dot-blots by densitometric scanning. Data are expressed as the ratio of specific mRNA to β-actin mRNA.

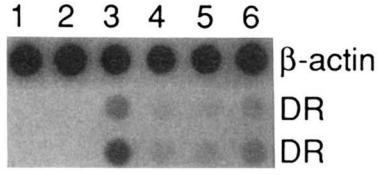

Figure 3. Time course of the TNF-α effect on IFN-γ induced HLA-DR mRNA expression in NLFB cells. Cells were incubated with IFN-γ (400 U/ml) for 72 h. TNF-α was added at 24 h before, 24 h after or simultaneously with IFN-γ. RNA dot-blot hybridization was performed as described. *Lane 1*, control cells incubated in medium alone; *lane 2*, TNF-α (50 ng/ml) alone (72-h incubation); *lane 3*, IFN-γ (400 U/ml) alone, *lane 4*, IFN-γ (400 U/ml) + TNF-α (50 ng/ml) added 24 h before IFN-γ; *lane 5*, IFN-γ (400 U/ml) + TNF-α (50 ng/ml) added simultaneously; *lane 6*, IFN-γ (400 U/ml) + TNF-α (50 ng/ml) added 24 h after IFN-γ. *Dots* in the bottom DR lanes were loaded with three times as much RNA than top DR lanes.

obtained in the different cells we hypothesized that the two mediators, IFN-γ and TNF-α, are synergistic on MHC class II expression in undifferentiated cells (HL-60 and THP-1). However, in mature cells (NLFB), TNF-α is antagonistic to the IFN-γ-induced class II expression. To test this hypothesis, the following series of experiments were performed.

HL-60 is a promyelocytic cell line that can be induced to differentiate into mature cells by a phorbol diester, TPA (32). Thus, HL-60 cells were induced to differentiated by TPA(10^{-7} M) for 5 days and surface expression of DR was tested after addition of IFN-γ, TNF-α or IFN-γ plus TNF-α for 48 h. As shown in Fig. 4, IFN-γ is capable of inducing DR expression both in undifferentiated HL-60 and in TPA-induced mature HL-60 cells. Although in undifferentiated HL-60 TNF-α alone induced class II expression (Fig. 4*A*), in TPA-induced mature HL-60, no such effect could be seen (Fig. 4*B*). Moreover, TNF-α also failed to synergize with IFN-γ in DR induction in the differentiated HL-60 cells. Similar results have been obtained in THP-1 cells, namely, TPA differentiated THP-1

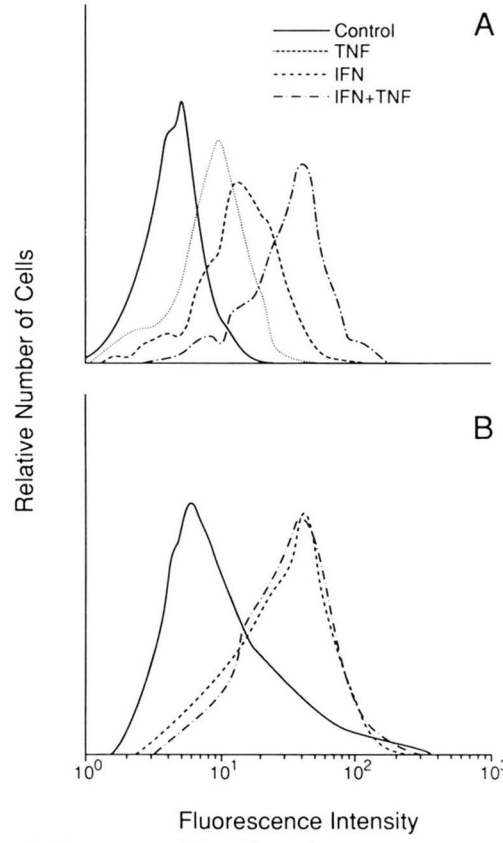

Figure 4. Comparison of the effects of IFN-γ, TNF-α, or both on HLA-DR expression on promyelocytic HL-60 cells (*A*) and TPA-induced differentiated HL-60 (*B*) analyzed by flow cytometry. Cells were incubated with or without TPA (10^{-7} M) for 5 days, washed, and followed by 2 days additional incubation with IFN-γ (1000 U/ml) or/and TNF-α (100 ng/ml). Flow microfluorimetry analysis was performed in the end of the incubation.

cells show a 32% reduction in DR expression by the combined treatment of TNF-α and IFN-γ compared to IFN-γ alone (data not shown).

Inasmuch as IFN-γ itself is a differentiation factor (33), we have treated THP-1 cells with IFN-γ for 5 days, after which the cells were washed and cultured for 48 h in control medium, medium containing IFN-γ, TNF-α, or both. As shown in Figure 5*B*, 2 days' incubation of cells without IFN-γ was sufficient to reduce DR expression to control levels, whereas continuous stimulation with IFN-γ maintained the HLA-DR expression. But most interestingly, TNF-α caused a very significant decrease in the HLA-DR expression in these differentiated cells.

Next, the effect of these mediators on DR expression in human fresh blood monocytes was compared to that in monocytes that were preactivated by IFN-γ for 5 days.

As shown in Figure 6, fresh monocytes from human peripheral blood can be induced to express DR by IFN-γ alone (48% increase in DR expression) and slightly (10% increase) also by TNF-α alone. However, TNF-α did not enhance the IFN-γ induced DR expression.

When the monocytes were activated by IFN-γ, they expressed a high level of DR. Further addition of IFN-γ did not potentiate HLA-DR expression (control/vs IFN) probably because the expression was maximal. This DR expression seemed to be irreversible in these activated cells, because culturing them for 2 additional days in medium without IFN-γ did not reduce the level of DR Ag on the surface of the cells. However, TNF-α alone de-

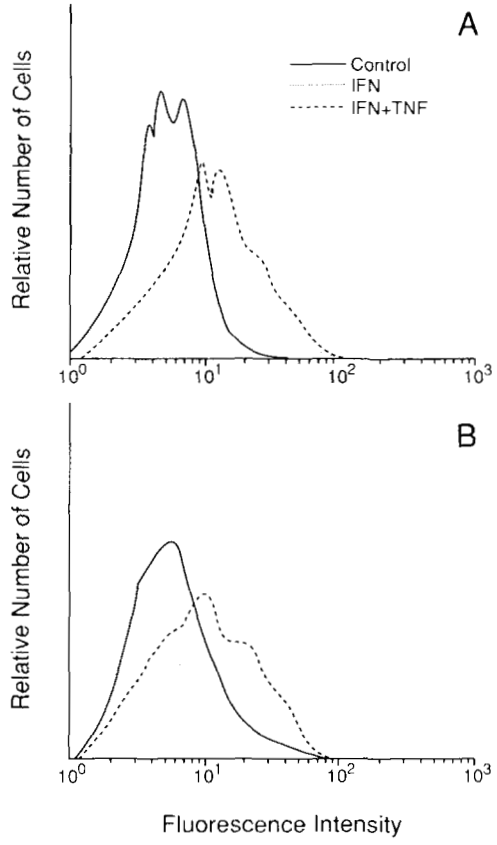

Figure 5. Comparison of the effects of IFN-γ, TNF-α, or both on HLA-DR expression in unstimulated monocytic THP-1 cells (*A*) and IFN-γ-induced differentiated THP-1 cells (*B*), and analyzed by flow cytometry. Cells were incubated in medium alone (*A*) or with IFN-γ (100 U/ml) (*B*) for 5 days, washed and followed by 2 days additional incubation with/without IFN-γ (100 U/ml) in the presence or absence of TNF-α (100 ng/ml).

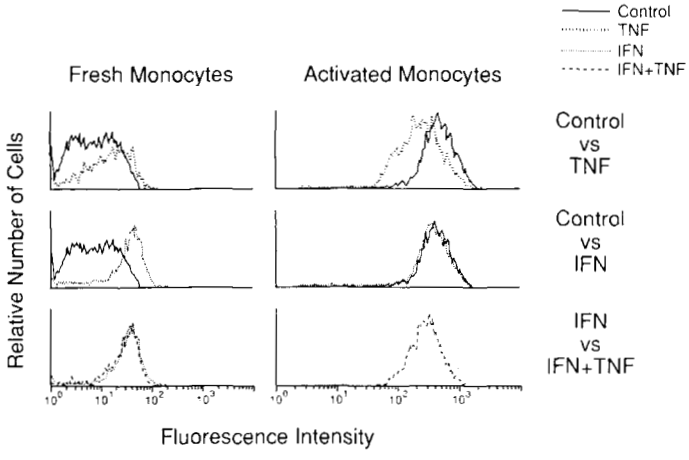

Figure 6. Comparison of TNF-α effects on HLA-DR expression between fresh human peripheral blood monocytes and activated monocytes. Fresh monocytes: cells were incubated with IFN-γ (100 U/ml) or/and TNF-α (100 ng/ml) for 2 days after separation from blood. Activated monocytes: cells were incubated with IFN-γ (100 U/ml) for 5 days, washed and followed by additional 2 days incubation with IFN-γ (100 U/ml) or/and TNF-α (100 ng/ml).

creased the constitutive DR expression by 50.6% in activated monocytes (control/vs TNF) and also antagonized the IFN-γ effect when added to the culture together (30% reduction compared to IFN-γ alone).

Effect of TNF-α on Ia expression induced by IFN-γ on bone marrow derived cells in different maturation stages. To confirm the relationship between the effect of TNF-α on the IFN-γ-induced class II expression and the maturation stage of the cells, we have tested Ia expression on murine bone marrow derived cells that were induced to mature in vitro by M-CSF (Fig. 7). M-CSF induces responsive bone marrow precursors into mature macrophages (34, 35). Fresh preparation of bone marrow cells depleted of adherent cells did not express Ia Ag before the incubation with M-CSF (day 0). However, after 2 days incubation, there are clearly two population of cells, one expressing Ia and a second that is Ia^- (Fig. 7*A*). All cells became Ia positive after 4 days incubation with M-CSF (Fig. 7*A*). Figure 7*B* shows that TNF-α slightly enhanced Ia expression in day 0 bone marrow cells (26% increase by TNF-α over IFN-γ) whereas IFN-γ alone did not induce Ia Ag expression. However, after 2 days incubation with M-CSF, these cells acquired the potential to respond to IFN-γ. Thus, IFN-γ significantly enhanced Ia expression and changed the two populations into one that was all Ia^+. Already at this stage (day 2) TNF-α showed slight down-regulation of the IFN-γ-induced Ia expression by 15%. Furthermore, in day 4 and 6 cells that were mature regarding Ia expression, TNF-α antagonized IFN-γ in Ia expression, causing a 30 and 50.2% reduction, respectively, on the level of Ia expression.

We have excluded the possibility that the number of TNF receptors may have changed during differentiation. As shown in Figure 7*C*, the TNF-α binding capability to bone marrow-derived cells remained constant during differentiation. Furthermore, using ^{125}I-TNF receptor binding assay, no significant variation in the number of receptors was noticed during differentiation (not shown). Thus the number of TNF receptors seems to be irrelevant to the antagonistic effect of TNF on Ia expression by IFN-γ.

In vivo effect of TNF-α on Ia expression by murine

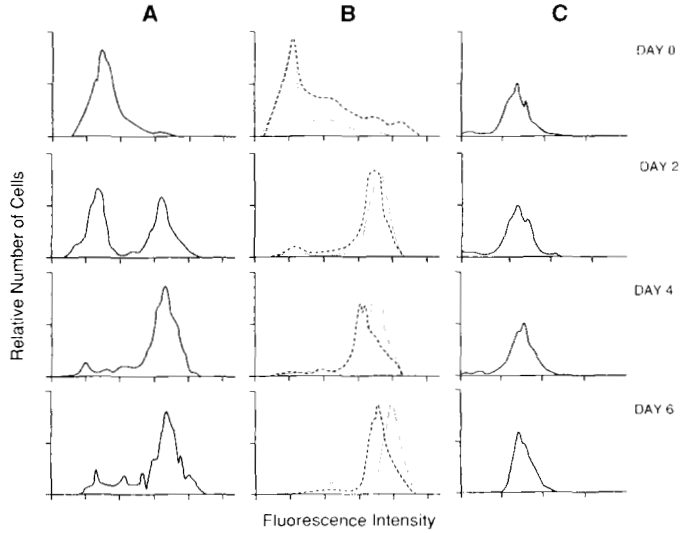

Figure 7. Effect of TNF-α on Ia expression induced by IFN-γ of bone marrow derived cells in different stages of maturation analyzed by flow cytometry. Fresh bone marrow cells depleted of adherent cells were induced to maturate in vitro by macrophage-CSF (25 U/ml). In *A*, cells at different stages of maturation (day 0, 2, 4, 6) were analyzed for Ia expression. In *B*, cells at day 0, 2, 4, 6 were washed and incubated with/without IFN-γ (40 U/ml) and/or TNF-α (100 ng/ml) for 48 h and analyzed by flow microfluorimetry for cell surface expression of Ia. In *B*, the profile for cells treated with IFN-γ (...) alone are superimposed with the profile for cells treated with IFN-γ plus TNF-α (– – –). In *C*, FITC-TNF binding to cells at different stages of differentiation was analyzed by flow microfluorimetry.

peritoneal macrophages. The down-regulation of IFN-γ-induced class II MHC by TNF-α is not only of academic interest, but may be relevant to the in vivo effects of TNF-α as exemplified by the following experiments.

Normal BALB/c mice or autoimmune (NZB × NZW)F1 mice received i.p. injections of rTNF-α (5 μg/injection) three times per week for a period of 3 wk. Control mice received PBS. Peritoneal exudate cells were obtained at the end of treatment and Ia expression on peritoneal macrophages was analyzed by flow cytometry. As shown in Figure 8, TNF-α treated (NZB × NZW)F1 mice (Fig. 8*A*) or normal BALB/c mice (Fig. 8*B*) show a significant decrease in Ia expression on their peritoneal macrophages compared to PBS controls. Moreover, in several separate experiments, 8 wk old (NZB × NZW)F1 mice have been treated with TNF-α (5 μg/injection) i.p. for 3 consecutive days. After this treatment peritoneal exudate cells obtained by peritoneal lavage, were incubated in vitro with different amounts of IFN-γ for 48 h and Ia expression was assayed by flow cytometry. As shown in Figure 9, peritoneal macrophages taken from TNF-α-pretreated mice have a significantly decreased response to IFN-γ as measured by expression of Ia, compared to PBS control.

DISCUSSION

The focus of the present studies was to analyze the role and the interactions between IFN-γ and TNF-α in the regulation of MHC class II expression, because both mediators have been suggested to play important roles in this tightly regulated process. Moreover, because class II MHC expression can be modulated by a variety of cytokines (14, 35–40) and physiologically, cells are likely to be exposed to combinations of these factors, it is attractive to assume that regulation of Ia expression involves the interaction of such cytokines.

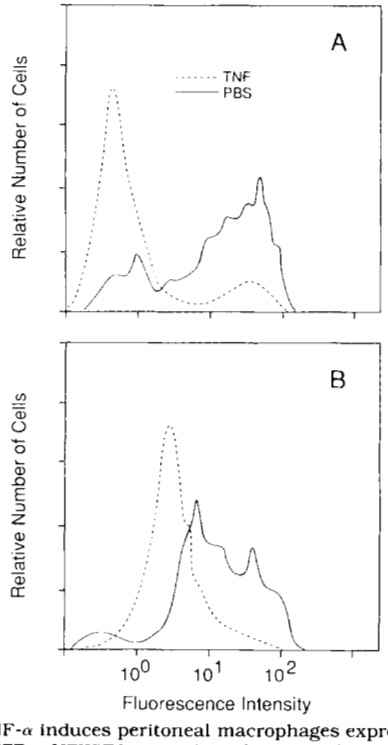

Figure 8. TNF-α induces peritoneal macrophages expressing reduced levels of Ia in (NZB × NZW)F1 mice (*A*) and in BALB/c mice (B). Eight wk old (NZB × NZW)F1 mice or BALB/c mice received i.p. injections of TNF-α (5 μg/injection) or PBS three times per week for a period of 3 wk. Mice were killed and their peritoneal cells were analyzed for Ia expression by flow cytometry.

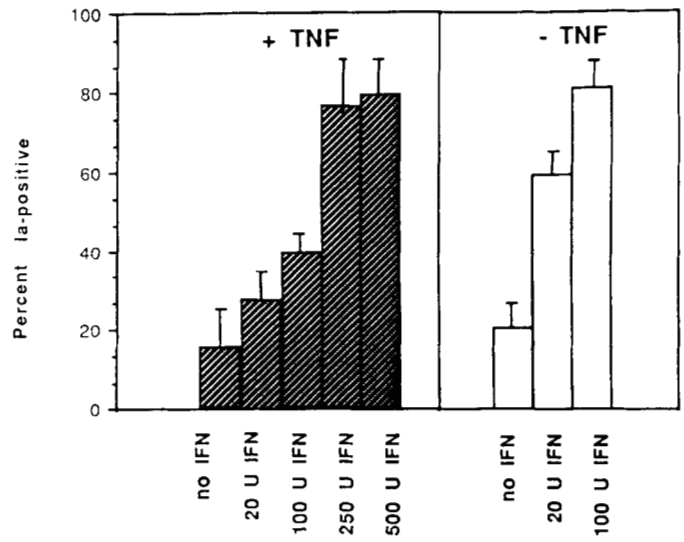

Figure 9. Reduced in vitro response of peritoneal macrophages to IFN-γ after TNF-α treatment in vivo. The 8 wk old (NZB × NZW)F1 mice were injected i.p. with 5 μg of TNF-α once a day for 3 days. Control mice received PBS. Mice were killed and their peritoneal adherent cells were cultured with different amounts of IFN-γ for 48 h and the proportion of macrophages expressing Ia was analyzed by flow microfluorimetry. As negative controls for comparison with all other samples, unstained peritoneal macrophages cultured for 3 days were used. These cells had a higher auto-fluorescence than freshly obtained peritoneal cells. Cells were also stained with a fluorescein-conjugated non-relevant anti-I-A antibody as negative controls. Data are represented as mean percent Ia^+ macrophages ± SD of four to five individually assayed mice per group. Macrophages were identified by MAC-1 positivity by flow microfluorimetry.

TNF-α has been previously shown both to synergize with IFN-γ and also to antagonize IFN-γ regarding regulation of DR and Ia molecules (22–26). Our findings are consistent with and also extend these previous observations. Thus, we have found that TNF-α down-regulates HLA-DR expression of human skin fibroblasts in a dose-dependent fashion, both on the mRNA and the protein level. However, in two monocytic cell lines HL-60 and THP-1, TNF-α synergizes with IFN-γ in up-regulating class II MHC expression.

We propose, that depending on the stage of differentiation and maturation of the cell, TNF-α might synergize or antagonize IFN-γ-induced regulation of MHC class II expression. Several lines of evidence have been provided to support this hypothesis. First, the human promyelocytic cell line, HL-60 (41, 42) and the human monocytic leukemia cell line, THP-1 (43) are known to be immature lines in terms of macrophage-like activities. However, both cell lines can be converted into mature cells using differentiation factors such as TPA or IFN-γ itself.

Thus, it has been shown that TPA treatment converts THP-1 cells into activated, mature macrophages that can phagocytize yeast and IgG-coated SRBC (32). Furthermore, this cell line has been suggested as a useful model for the study of the mechanisms of maturation from monocytes to macrophages (32).

TNF-α enhanced IFN-γ-induced MHC class II expression on both HL-60 (Fig. 4*A*) and THP-1 (Fig. 5*A*). However, although TNF-α alone induced HLA-DR expression only in HL-60, both the spontaneous and the synergistic effect of TNF-α with IFN-γ on class II expression was eliminated after TPA treatment. Similarly, differentia-

tion of THP-1 cells, either by IFN-γ treatment or TPA treatment, reversed the effect of TNF-α from synergistic to antagonistic on IFN-γ-induced DR expression. Similar results were obtained when we compared fresh human blood monocytes to activated monocytes (Fig. 6). Finally, bone marrow cells were induced to differentiate into mature macrophages in vitro by macrophage-CSF (34, 35). Our data indicate that TNF-α antagonized IFN-γ-induced Ia expression in a maturation-dependent fashion. Thus, in day 0 bone marrow cells, TNF-α enhanced Ia expression by 26%, whereas in M-CSF-treated bone marrow cells for 2, 4, and 6 days, TNF-α decreased Ia expression by 15, 30, and 50%, respectively.

An analysis of the literature of TNF-α effect on IFN-γ-induced class II expression on the basis of the maturation and differentiation stage of the cells supports our hypothesis. Thus, TNF-α increases IFN-γ-induced MHC class II expression in a murine myelomonocytic cell line (22), several human undifferentiated tumor cells (23), human monocytic cell lines (24), and embryonic mouse spinal cord tissue (44). However, TNF-α decreases the expression of Ia on thyoglicolate-induced peritoneal macrophages (25), mature endothelial cells (26), human keratinocytes (Dr. S. Lee, personal communication), human skin fibroblasts, differentiated monocytic cell lines, and differentiated macrophages. Taken together, these results suggest a rational explanation for the conflicting reports regarding the effect of TNF-α on the IFN-γ-induced class II MHC expression.

The reports by Pujol-Borrell et al. (45) and Wright et al. (46) that pancreatic β cells can be induced to express HLA-DR and Ia by INF-γ and TNF-α seem to contrast with our hypothesis. However a more carefull analysis of the data suggest that only a portion of β cells are class II^+ (45). Furthermore, Campbell et al. (47) found that only 30 to 40% of normal β cells are susceptible to HLA-DR and DQ or Ia induction by IFN-γ and TNF-α and the rat β cell tumor line RIN-m5F was not induced to express class II MHC proteins by this treatment. These authors conclude that the differentiation state of the β cells may determine its susceptibility to induction of class II MHC proteins by IFN-γ and TNF-α (47).

The down-regulation of the IFN-γ-induced class II MHC expression by TNF-α in differentiated, mature, and activated cells may not be only of academic interest, but may actually be relevant to the physiologic roles of this cytokine in vivo. Thus, we have shown that in vivo treatment of normal mice or autoimmune mice caused a significant reduction in the level of Ia surface expression on their peritoneal macrophages. However, it should be emphasized that we do not really know whether TNF-α treatment in these experiments actually caused down-regulation of Ia expression on those macrophages or whether TNF-α induced in the peritoneum a new population of macrophages that express a lower level of Ia molecules.

The second set of in vivo experiments, in which peritoneal macrophages taken from TNF-α-treated mice show a significantly decreased response to IFN-γ as measured by cell surface expression of Ia molecules, supports the notion that TNF-α actually antagonizes the IFN-γ-induced increase in Ia expression on peritoneal macrophages rather than just inducing a different cell population in the peritoneum. It is reasonable to assume that Ag- presenting capability is a property of mature, differentiated, and activated cells rather than primitive or immature cells. Thus the down-regulation of IFN-γ-induced class II MHC expression by TNF-α might be much more relevant biologically than the synergistic effect on undifferentiated cells.

Furthermore these findings may resolve the apparent paradoxical in vivo effects of TNF-α and IFN-γ in the autoimmune (NZB × NZW)F1 mice. We have previously shown that IFN-γ can enhance the development of lupus nephritis in (NZB × NZW)F1 mice (48). However, treatment of such mice with TNF-α significantly delayed the development of the disease (49). Thus, the apparent opposite effects of TNF-α and IFN-γ in regulation of class II MHC molecules in this in vivo system may imply that regulation of Ia expression is the mechanism by which TNF-α or IFN-γ affect the development of lupus nephritis in (NZB × NZW)F1 mice. However, because the functional significance of inappropriate expression of MHC class II Ag in initiating or propagating autoimmune disease is unknown (50) and both IFN-γ and TNF-α have multiple biologic activities, the actual mechanism by which TNF-α and IFN-γ affect the development of lupus nephritis in (NZB × NZW)F1 mice remains unresolved.

A second issue that remains unresolved is the mechanism(s) by which TNF-α mediates its effect regarding class II MHC expression. There is the possibility that the effect of TNF-α is mediated by other factors such as IFN-α/β (37) and/or PG (51). Inasmuch as TNF-α is part of a complex interactive network of cytokines and TNF-α can induce several other factors including IL-6 (52), PGE2 (53), and granulocyte-macrophage-CSF (54), this possibility should be considered. In this regard, we have recently shown that neither IL-6 nor PGE2 inhibits IFN-γ-induced MHC class II expression (55).

Conversely, the data presented suggest that differences in gene expression during differentiation of the cell may be relevant to class II MHC expression by IFN-γ and TNF-α. It is conceivable to assume that some genes relevant to this biologic function are activated or inactivated during transfer from immature to differentiated stage of cells.

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