Brief Definitive Report

INTERFERON β1, AN INTERMEDIATE IN THE TUMOR NECROSIS FACTOR α–INDUCED INCREASED MHC CLASS I EXPRESSION AND AN AUTOCRINE REGULATOR OF THE CONSTITUTIVE MHC CLASS I EXPRESSION

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In inflammatory reactions an increased expression of HLA class I and class II antigens is observed (1). This enhanced expression is expected to play a role in the activation of both MHC class I– and class II–restricted T cells. In vitro, the mediators interferon β (IFN-β), tumor necrosis factor α (TNF-α), and IFN-γ induce an increase in the MHC class I expression on human umbilical venal endothelial (HUVE) cells and on human fibroblasts (2). Recombinant TNF-α has been shown to increase the mRNA level and the surface expression of MHC class I antigens via a newly synthesized protein intermediate, as was demonstrated in experiments using a protein synthesis inhibitor (2). A candidate for this intermediate is IFN-β1, since IFN-β1 induces an increase in MHC class I expression on HUVE cells and on human fibroblasts (2). It has been suggested, however, that IFN-β2 is the possible intermediate responsible for the increased MHC class I expression induced by TNFα (3).

We studied the putative role of IFN-β1 in the increase in MHC class I expression and the antiviral activity induced by TNF-α on HUVE cells and on human fibroblasts. Our results demonstrate that IFN-β1 plays the major role in the rTNF-α-mediated increase in MHC class I expression and the antiviral activity. Furthermore, it was shown that IFN-β1 is involved in the autocrine regulation of the constitutive MHC class I expression on HUVE cells and human fibroblasts.

Materials and Methods

Cells. HUVE cells were isolated by selective collagenase digestion of the umbilical vein. The cells were cultured in fibronectin-coated tissue culture flasks in RPMI 1640 (Gibco Europe, Paisley, Scotland), supplemented with 20% heat-inactivated human serum, 50 μg/ml heparin, endothelial cell growth supplement (30 μg/ml; Collaborative Research, Lexington, MA), and antibiotics. Human fibroblasts were cultured in RPMI 1640 supplemented with 10% heat inactivated FCS and antibiotics.

Reagents. rTNF-α was kindly provided by Dr. F. Frickel (Knoll Ag, Ludwigshafen, Federal Republic of Germany) in cooperation with Biogen (Geneva, Switzerland). rIFN-

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β1 was kindly provided by Prof. Bujard (Hoffman-LaRoche, Basel, Switzerland), and did not show hybridoma growth factor activity at antiviral doses as high as 1,000 U/ml. The 26 kD protein, also designated as hybridoma growth factor (HGF) (4), or IFN-β2 (5) or BSF-2 (B cell stimulatory factor) (6) was obtained from MG-63 osteosarcoma cells induced with IL-1 and purified by adsorption to controlled-pore glass beads and by antibody chromatography to a specific activity of 30×10^6 26-kD U/mg (manuscript in preparation). One unit of 26 kD protein was defined as the amount required to obtain half-maximal growth of factor-dependent hybridoma cells. The biologic activity of the 26 kD preparations used in our experiments showed 250,000 U/ml of hybridoma growth factor activity, while <10 U/ml of antiviral activity could be detected. Furthermore, rBSF-2 (6), kindly provided by Prof. T. Hirano (Osaka, Japan) was used.

Antisera. An anti-TNF-α antiserum with TNF-α-neutralizing capacity was obtained by immunizing rabbits with human rTNF-α. The antiserum showed a specific reaction in immunoblot with a 17 kD product in macrophages corresponding with the reaction with rTNF-α. Anti-IFN-β1 was obtained by immunizing a goat with highly purified IFN-β1 (10^8 U/mg); this antiserum had a neutralizing titer of more than 1:500,000 against IFN-β1 (10 U/ml), but virtually no activity against 26 kD protein (titer <1/100). Rabbit anti-dog IgG was purchased from Miles Laboratories, Elkhart, IN.

Induction and Detection of MHC Class I Antigens. Cells were incubated for 4 d in culture medium supplemented with the reagents in the concentrations as indicated. Mixtures of reagents with antibodies were preincubated for 4 h before adding them to the cells.

The expression of MHC antigens was studied with an indirect immunofluorescence technique. MHC class I antigens were quantitated by staining with mAb W6/32, followed by incubation with FITC-labeled antiserum against mouse Ig. Fluorescence was assessed with a FACS IV cell sorter. The data were expressed in relative fluorescence intensity units (RFI), according to the formula: $\text{RFI} = \sim 10^{[0.0198\ \Delta(\text{experimental cells})]} / 10^{[0.0198\ \Delta(\text{control cells})]}$. The factor 0.0198 represents conversion of log to linear fluorescence distributions; Δ(experimental cells) and Δ(control cells) being the difference in modal log green fluorescence channel between cells, with and without mAb anti-class I.

Assay for Antiviral Activity. Antiviral activity of rTNF-α, rIFN-β1, and 26 kD protein was determined on diploid fibroblasts and on HUVE cells using a cytopathic effect reduction assay. Vesicular stomatitis virus was used as a challenge and cells were stained with crystal violet to measure cytopathic effect. The potency of IFN-β1 was expressed in international units (U/ml).

Results

rTNF-α Induces an Increase in MHC Class I Expression on HUVE Cells and on Human Fibroblasts via IFN-β1. Subconfluent HUVE cells and human fibroblasts were cultured for 4 d with different concentrations of rTNF-α in the presence or absence of either an antiserum specifically directed against IFN-β1, or against rTNF-α (data not given). As a control an irrelevant rabbit anti-dog IgG antiserum was included (data not given). The data in Table I demonstrate that the increase in MHC class I expression is dependent upon the concentration of rTNF-α, reaching a plateau value at ~30 ng/ml rTNF-α. The plateau value varied per experiment from 1.7 to 4.2 on HUVE cells and human fibroblasts as expressed in RFI units. The addition of anti-IFN-β1 to the cultures reduced this increase markedly, suggesting that IFN-β1 in the intermediate messenger. The inhibition of the rTNF-α-induced increase in MHC class I expression by anti-IFN-β1 cannot be attributed to a nonspecific serum effect, since an anti-dog IgG serum did not interfere with the MHC class I expression. Antiserum directed against rTNF-α blocked the rTNF-α effect, indicating that the increase in MHC class I expression is attributable to rTNF-α and not to possible contaminants present in the preparation.

Effect of rIFN-β1 and IFN-β2 (26 kD and rBSF-2) on MHC Class I Expres-

TABLE I

Effect of Anti-IFN-β1 on the rTNF-α-induced Increase in MHC Class I Expression on HUVE Cells and Human Fibroblasts

TNF-α	HUVE cells				Fibroblasts			
	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	−*	+*	−	+	−	+	−	+
ng/ml								
0	1.0	1.0	1.0	0.7	1.0	0.9	1.0	0.9
3	1.8	1.3	1.6	0.9	1.7	0.9	1.8	1.0
10	3.3	1.5	1.9	0.8	1.7	1.1	2.2	1.2
30	3.5	1.4	1.9	1.1	4.0	0.9	4.2	1.6

Data are expressed in RFI as described in Materials and Methods.
* Subconfluent cells were cultured for 4 d in the absence (−) or presence (+) of antiserum directed against IFN-β1.

TABLE II

Effect of rIFN-β1 and IFN-β2 (26 kD and rBSF-2) on MHC Class I Expression in Subconfluent Growing HUVE Cells and Fibroblasts

IFN*	HUVE cells			Fibroblasts		
	IFN-β1	26 kD	rBSF-2	IFN-β1	26 kD	rBSF-2
U/ml						
10	1.4‡	—	—	1.7	—	—
50	1.8	1.0	1.0	1.7	1.0	1.0
150	2.0	1.0	1.0	2.3	1.1	0.9
450	1.6	1.2	0.9	2.2	1.4	1.0
1,350	—	1.1	0.8	—	1.5	1.0
4,050	—	1.3	—	—	1.2	—

* IFN-β1 is expressed in antiviral units per milliliter; 1 U/ml = 0.5 pM; IFN-β2 is expressed in hybridoma growth-promoting units: 26 kD, 1 U/ml = 0.4 pM; rBSF-2, 1 U/ml = 0.3 pM.
‡ Data are expressed in RFI (see Materials and Methods).

sion. IFN-β1 and IFN-β2 (either highly purified 26 kD or rBSF-2) were tested for their effect on MHC class I expression on HUVE cells and on human fibroblasts. In Table II the results show that IFN-β1 increases the MHC class I expression. Although purified natural IFN-β2 (26 kD) showed inconsistently a slight effect on the expression of MHC, highly purified IFN-β2 (rBSF-2) did not enhance MHC class I expression even at high concentrations.

Antiviral Effect of rTNF-α on HUVE Cells and on Human Fibroblasts Is Abrogated by Anti-IFN-β1. The role of IFN-β1 and the 26 kD protein in the antiviral effect of rTNF-α and IL-1 on fibroblastoid cells has been investigated in a separate study (7). In view of our findings with rTNF-α on MHC class I expression in HUVE cells and fibroblasts, we also studied the possible antiviral activity of rTNF-α on those cells. Table III shows that rTNF-α and IFN-β1 have an antiviral effect on HUVE cells, similar to that on diploid fibroblasts. Moreover, the antiviral activity of rTNF-α on HUVE cells was also completely neutralizable by antibody against IFN-β1. This finding indicates that IFN-β1 is an important mediator of the antiviral effect of rTNF-α in the same cell systems in which it plays a role in rTNF-α-induced MHC class I expression.

IFN-β1 Plays a Role in the Constitutive MHC Class I Expression. As shown in Table I, anti-IFN-β1 not only inhibited the rTNF-α effect, but it also caused a decrease in the class I expression that occurs in the absence of rTNF-α. This

TABLE III

Antiviral Effect of rTNF-α on Diploid Fibroblasts and HUVE Cells and Its Neutralization by Specific Antibody against IFN-β1

Cytokine tested	Antibody added		Antiviral activity on:	
	Specificity	Reciprocal dilution	Fibroblasts	HUVE cells
			U/ml	
TNF-α (1,400 ng/ml)	—	—	1,000	640
	Anti-TNF-α	50	<32	64
	Anti-IFN-β1	3,000	<32	<10
IFN-β1	—	—	10,000	6,400
	Anti-TNF-α	50	16,000	5,000
	Anti-IFN-β1	3,000	10	<10

A constant amount of antiserum was added to each dilution of the antiviral assay, and reaction mixtures were incubated at 37°C for 2 h before transfer to the test cells.

TABLE IV

Effect of rIFN-β1 and Anti-IFN-β1 on MHC Class I Expression by Confluent HUVE Cells and Fibroblasts

Exp.	HUVE cells		Fibroblasts	
	rIFN-β1*	Anti-IFN-β1‡	rIFN-β1	Anti-IFN-β1
1	1.1	0.6	1.7	0.6
2	1.6	0.6	2.0	0.5
3	2.6	0.8	1.2	0.7
4	1.5	0.5	1.9	0.9
5	1.5	0.5	1.1	0.8
Mean ± SE	1.66 ± 0.25	0.6 ± 0.05	1.58 ± 0.18	0.7 ± 0.07

* 300 U/ml rIFN-β_1 was added to the cultures.
‡ Antiserum directed against IFN-β1 was incubated at a final dilution of 1:3,000.

prompted us to study the role of autocrine IFN-β1 in the regulation of the constitutive MHC class I expression. A pilot experiment demonstrated that the effect of anti-IFN-β1 on the constitutive MHC class I expression was most marked in aged cells, i.e., cells kept in culture in the same medium for several days after confluency was reached. Therefore, we compared the influence of exogenous added rIFN-β1 and anti-IFN-β1 on confluent cells without changing the culture medium. Both in fibroblasts and in HUVE cells, anti-IFN-β1 added to confluent cultures reduced the MHC class I expression to ~65% of the normal spontaneous expression (Table IV), indicating that IFN-β1 is involved in the expression of the constitutive MHC class I antigens on these cells. Addition of rIFN-β1 resulted in an increase of MHC class I expression to ~160% of spontaneous expression (Table IV). Control antibodies did not affect the IFN-β1-induced increase in MHC class I expression (data not shown).

Discussion

In accordance with others (2, 8), we show here that rTNF-α induces an increase in MHC class I expression on HUVE cells and human fibroblasts. This increase can be inhibited by antibodies specifically neutralizing human IFN-β1.

Similarly, the antiviral activity mediated by rTNF-α that is shown to occur not only in tumor cells (9, 10) and in fibroblasts (3, 7), but also in endothelial cells can be completely abrogated by anti-IFN-β1. These results indicate that IFN-β1 is involved in the rTNF-α-mediated increase in MHC class I expression and antiviral activity.

Addition of rIFN-β1 to HUVE cells and fibroblasts increased the MHC class I expression. Antiserum directed against IFN-β1 caused a decrease of the MHC class I expression by the cells not treated with rTNF-α or rIFN-β1. This effect was most pronounced in confluent cultured cells, probably because of an internal pool of IFN-β1. Autocrine IFN-β1 is apparently involved in the constitutive MHC class I expression. Thus, the mechanism by which rTNF-α acts upon the cells is not a new mechanism, but rather an enhancement of the physiological autocrine regulation of HLA gene expression.

Collins et al. (2) described that the effect of rTNF-α on the MHC class I expression was regulated via a mediator. Our studies strongly indicated that IFN-β1 is the mediator responsible for this process. Others, however, suggested IFN-β2 to be the intermediate (3, 8). They observed high levels of IFN-β2 mRNA, whereas they failed to detect IFN-β1 mRNA in TNF-α-treated fibroblasts. Experiments with IFN-β2 (26 kD protein and rBSF-2) showed that even high doses (>4,000 U/ml) did not or only very slightly affect the MHC class I expression and lacked antiviral activity on HUVE cells and fibroblasts. Although the possibility cannot be excluded that IFN-β2/26 kD/rBSF-2 protein plays a limited role in the rTNF-α-mediated increase in MHC class I expression and antiviral activity, our results indicate that IFN-β1 is the major factor responsible for these effects of rTNF-α. Autocrine secretion of IFN-β by the tumor cell line U937 triggers the expression of MHC class I antigens (11). Our data provide evidence that this phenomenon is not restricted to that monocytic tumor cell line, but is also functional in normal HUVE cells and human fibroblasts. It is likely that the constitutive MHC class I expression in various cell types is at least in part regulated via autocrine IFN-β1. The autocrine regulation of MHC class I antigen expression combined with virus protection is a functional strategy, since the MHC class I expression plays a role in the recognition and destruction of virus-infected cells by MHC class I–restricted T lymphocytes, and simultaneously the IFN-β1 protects the cell against virus infection. Furthermore, the TNF-α-induced upregulation of the MHC class I expression may be of importance for the recognition of tumor cells by T lymphocytes. The absence or reduction in number of MHC class I molecules (12) is a mechanism by which tumor cells may escape from destruction by the immune system. Alternatively, the enhancement of MHC class I expression may rescue such tumor cells from the activity of natural killer cells, since these cells were suggested to preferentially kill cells with a deleted or reduced expression of MHC antigen (13). Thus, TNF-α and the interferons provide the cells with virus protection as well as with enhanced MHC class I expression, resulting in a double surveillance.

Summary

In conclusion, our observations indicate that the constitutive MHC class I expression is regulated by autocrine production of IFN-β1. TNF-α acts as an

enhancer of the autocrine production of IFN-β1, and consequently as an enhancer of the MHC class I expression and viral protection.

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