Brief Definitive Report

EXPRESSION OF INTERLEUKIN 2 RECEPTORS AND BINDING OF INTERLEUKIN 2 BY GAMMA INTERFERON-INDUCED HUMAN LEUKEMIC AND NORMAL MONOCYTIC CELLS

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There is growing evidence to suggest a role for the T cell lymphokine gamma interferon (IFN-γ) in the regulatory biology of cells of the monocyte/macrophage lineage. IFN-γ induces differentiation of immature myeloid cells along the monocytic pathway and induces or enhances expression of class II HLA antigens during this process (1). Upon exposure to IFN-γ, immature and mature monocytic cells acquire tumoricidal activity (2) and express new receptors for Fc fragments that bind antibody-coated particles, and so initiate phagocytosis. In addition, IFN-γ inhibits monocyte migration in a manner similar to macrophage migration inhibitory factor (3), and inhibits growth of myeloid progenitor cells, particularly those destined to form granulocyte colonies (4). In the present report, we show by immunofluorescence and biochemical identification that IFN-γ induces surface expression of receptors for interleukin 2 (IL-2) by normal human monocytes and by the monocytic and promyelocytic cell lines U937 and HL60. These results suggest the possible involvement of an IL-2 receptor (IL-2R) system on human monocytic cells in monocyte/T cell interaction.

Materials and Methods

Cells Analyzed for IL-2R Expression. Human myeloid leukemia cell lines KG1, HL60, U937, and HEL were maintained in RPMI 1640 medium with 10% fetal calf serum. Mononuclear cell suspensions from acute myeloblastic leukemia (AML) and normal bone marrow (NBM) specimens were prepared by Ficoll-Hypaque density gradient centrifugation. NBM progenitor cells enriched 80–100-fold for myeloblasts were obtained using an immune rosette technique previously described (5). Erythroid precursor cells were prepared similarly from human fetal liver mononuclear cells. B cell–enriched populations were obtained from normal spleen lymphocytes by depletion of T cells and monocytes. Normal T cells were prepared by E rosetting. Monocytes were isolated from E⁻ cells by two plastic adherence steps. Granulocytes were obtained using dextran sedimentation.

Induction of IL-2R Expression. To induce IL-2R expression, cells were cultured at 1 × 10^6 cells/ml in flat-bottom tissue culture plates (35 × 14 mm) in the presence of 10–1,000 U/ml of recombinant IFN-γ (kindly provided by Dr. Steven Reich, Biogen, Cambridge, MA) for a period of 12–96 h.

In selected experiments other compounds known to affect differentiation of myeloid/ monocytic cell lines were added for 68 h to U937 and HL60 cultures, including phyto- hemagglutinin (PHA) leukocyte-conditioned medium (PHA-LCM, 10% vol/vol), recom- binant alpha IFN (500 U/ml) (Hoffman-La Roche, Inc., Nutley, NJ), prostaglandin E_2 (10^{-6} M), retinoic acid (10^{-6} M), dimethyl sulfoxide (DMSO) (1.1% vol/vol), 1,25(OH)$_2$ vitamin D3 (10^{-8} M) (Hoffman-La Roche, Inc.), and cAMP (10^{-3} M) (Sigma Chemical Co., St. Louis, MO).

Monoclonal Antibodies.   Anti-TAC (6) antibody was a gift from Dr. T. Waldmann. All other antibodies used were produced in the Division of Tumor Immunology, including anti-T3, -T11, -T9, -B1, -B4, and -HLA-DR (9–49), MY4, Mo2, and anti-IL-2R1. The characterization of these antibodies has been previously reviewed (7).

Immunofluorescence Staining.   For one-color staining, 1 × 10^6 cells were incubated for 30 min at 4°C with the appropriate monoclonal antibody or irrelevant isotype-identical control antibody. If antibodies were not directly fluorescein conjugated, a second incu- bation was performed for 30 min at 4°C with fluorescein-conjugated goat anti–mouse Ig (Tago, Inc., Burlingame, CA). Washing medium contained 10% human AB serum.

For dual fluorescence studies, IFN-γ-treated monocytes were incubated with biotin- conjugated anti-Mo2 and a fluoresceinated anti-IL-2R1 (8). After washing, cells were developed with avidin-conjugated Texas Red (Molecular Probes, Junction City, OR). Cells were analyzed using a dual laser flow cytometer (EPICS V; Coulter Electronics, Hialeah, FL).

Iodination, Immunoprecipitation, and SDS-PAGE.   U937 cells treated for 60 h with 500 U/ml IFN-γ were externally labeled with ^{125}I using the lactoperoxidase-catalyzed method (10). Immunoprecipitates with anti-TAC antibody were analyzed by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE).

Effect of Recombinant IL-2 (rIL-2) on IFN-γ-primed U937 Cells.   To determine whether rIL-2R bound to induced IL-2R, the proliferative response of rIFN-γ primed U937 to rIL-2 was tested. U937 cells (1 × 10^6/ml) were cultured in the presence of 500 U/ml rIFN-γ (concentration of IFN-γ was determined by Biogen). After 48 h, cells were harvested by vigorous pipetting, transferred to 96-well, round-bottom plates (Linbro Chemical Co., Hamden, CT) and cultured for another 48 h in the presence of various dilutions (100, 500, 1,000 U/ml) of rIL-2 at 5 × 10^4 cells/ml. 12 h before cell harvest some of the cultures were pulsed with 1 μCi [^{3}H]thymidine ([^{3}H]TdR). To study adsorption of added rIL2 by IL-2R-expressing U937 cells, we assayed supernatants from nonpulsed cultures for IL-2 using IL-2-dependent T cells as described (9). The proliferative response to IL-2 was measured as [^{3}H]TdR uptake.

Results and Discussion

IL-2 stimulates in vitro proliferation of activated T and B cells (6, 10, 11) by acting through specific cell membrane receptors that have been identified as p60–65 antigens by anti-receptor monoclonal antibodies (6, 8). We show that not only T and B cells but also normal human peripheral blood monocytes and leukemic cell lines (U937, HL60) capable of monocytic differentiation, express IL-2 surface receptors when exposed to the T cell lymphokine IFN-γ.

Exposure of U937 cells to rIFN-γ (500 U/ml) induced expression of binding sites for anti-IL-2R antibody 24–48 h after initiation of cultures (Table I). Identification of these binding sites as IL-2R was confirmed by immunoprecipi- tation of the same 60–65 kD protein from surface-labeled U937 cells as from PHA-activated T cells (Fig. 1). Also, rIL-2-induced U937 cells could adsorb IL- 2 biological activity but noninduced U937 cells could not (Table II). The time course of induction of IL-2R paralleled the induction of class II HLA antigens, suggesting de novo synthesis rather than the unmasking of cryptic receptors. In a survey of hematopoietic cells (Table III), the induction of IL-2R by IFN was

TABLE I

Induction of IL-2R on U937 Cells

Dose response (48 h culture)

rIFN-γ	Anti-IL-2R1[+]	Recovery*
U/ml	%	%
0	2.0	100
50	17.1	98
500	52.7	76
250	32.6	81
1,000	41.6	49

Kinetics of IL-2R expression (500 U/ml rIFN-γ)

Time	Anti-IL-2R1[+]	Recovery
h	%	%
0	2.0	100
12	7.2	96
24	16.4	86
48	52.7	76
96	43.3	61

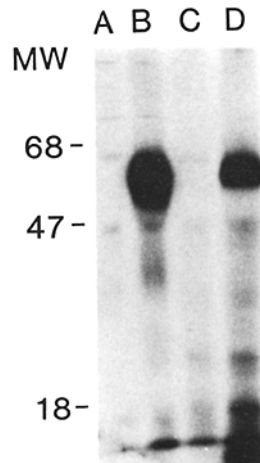* Percent of initial cell number in culture.

FIGURE 1.   SDS-PAGE analysis of immunoprecipitates obtained with anti-TAC antibody on PHA-activated T cells (*B*) and IFN-γ (48 h, 500 U/ml)-treated U937 cells (*D*) under reducing conditions. *A* and *C* represent control immunoprecipitates from the same cells using an unreactive control antibody (anti-glycophorin A, clone 39).

TABLE II

Adsorption of IL-2 by U937 Cells

	[3H]TdR incorporation of IL-2-dependent T cells
	cpm
Uninduced U937 cells*	5,100 ± 470
IFN-γ-induced U937 cells	200 ± 35

* U937 cells (1 × 10^6/ml) were cultured in the presence or absence of rIFN-γ for 48 h, washed, and recultured in fresh medium for an additional 48 h in the presence of rIL2 (500 U/ml). Culture supernatants were then removed and assayed for the presence of remaining IL-2 on IL-2-dependent human T cells. For this, T cells (plus 5% macrophages) previously cultured for 7 d with PHA (0.5 mg/ml) were recultured in 96-well plates (5 × 10^4/well, 0.2 ml total volume per well) in the presence of 20% (vol/vol) of the supernatants.

TABLE III

Effect of rIFN-γ (48 h, 500 U/ml) on IL-2R Expression of Various Cell Types

Leukemic cells:	Percent cells stained with anti-IL-2R1			
	HL60	KG-1	AML (M1, M2) (n = 3)	AML (M4, M5) (n = 5)
Noninduced	1.4	64.0	0	0–1.7
rIFN-γ induced	37.6	63.3	0–1.3	5.7–37.6

Normal cells:	Granulo-cytes	Monocytes	T cells	B cells	Myeloblasts	Erythroid progeni-tors
Noninduced cells	0	0	0	0	0	0
rIFN-γ induced cells	0	62.9	1.5	0	4.0	1.7

TABLE IV

Effects of Various Differentiation-inducing Compounds (48 h exposure) on Myeloid Cell Lines

	Nontreated			rIFN-γ			rIFN-α			PHA-LCM			PGE2		
	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1
							%								
Anti-IL2R⁺	2.0	1.4	64.0	52.7	37.6	63.3	1.9	1.5	64.2	53.8	37.5	63.7	1.7	1.4	62.9
HLA-DR⁺ (9–49)	9.8	19.1	92.4	51.4	76.9	94.8		ND		54.0	75.3	97.1	9.0	19.1	90.3
MY4⁺	10.1	8.2	6.1	65.4	67.2	6.4		ND		ND	66.6	5.4	9.9	8.0	6.0

	PGE2 + rIFN-γ			1,25(OH)2 vitamin D3			DMSO			cAMP			Retinoic acid		
	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1	U937	HL60	KG1
							%								
Anti-IL2R⁺	30.5	21.1	63.8	9.3	2.7	60.8	1.1	1.2	60.1	2.0	1.9	ND	1.9	1.5	62.9
HLA-DR⁺ (9–49)	24.9	65.0	ND	38.0	ND			ND		12.7	ND			ND	
MY4⁺	ND	ND	ND	78.9	ND			ND		40.1	ND			ND	

Interferon concentration was 500 U/ml. ND, not done. See text for concentrations used. Recovery of viable cells in each fraction was >75%.

restricted to monocytic cells under the conditions used in this study, except for the myeloblast cell line KG-1.

IL-2R expression on the myeloid cell lines U937 and HL60 was associated with induction of monocytic differentiation as evidenced by the monocytoid morphology, expression of the monocyte membrane antigen MY4 (Table IV). Compounds that are known to cause differentiation of HL60 cells into granulocyte cells (DMSO and retinoic acid) failed to induce IL-2R on HL60 and U937 (Table IV). Similarly, granulocytes (Table III) and normal or leukemic myeloblasts (fresh M1 or M2 AML samples) could not be induced to express IL-2R by IFN-γ. However, more "monocytic" AML samples (M4, M5) could be induced. Further, IFN-γ-induced IL-2R expression of U937 cells was restricted to cells that became adherent during the culture (data not shown). Prostaglandin E_2 (PGE2), known to inhibit monocyte differentiation and class II antigen expression (12) partially inhibited the IFN-γ-induced IL-2R expression on U937 cells (Table IV). However, other compounds that promote macrophage-like differentiation of U937, such as 1,25(OH)2 vitamin D3, did not cause IL-2R expression on U937 cells (Table IV) suggesting a specific role for IFN-γ in inducing IL-2R on macrophage-like differentiated cells.

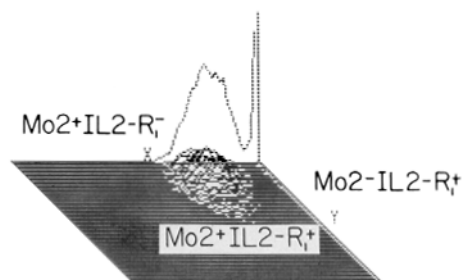Since IL-2 induces proliferation of IL-2R⁺ T and B cells, the function of IL-

FIGURE 2.   Dual color fluorescence analysis of IL-2R expression on IFN-γ (48 h, 500 U/ml)-treated peripheral blood monocytes. Mo2 antigen was detected with biotin-conjugated anti-Mo2 (x axis), and IL-2R detected with fluorescein IL-2R1 monoclonal antibody (y axis).

2R on U937 cells was investigated. IFN treatment of U937 cells induced a dose-dependent inhibition of [³H]TdR incorporation as expected. Addition of IL-2 to IGN-γ-treated U937 cells modestly increased [³H]TdR incorporation in a dose-dependent manner, but never to greater than starting levels (data not shown). This IL-2 effect could be blocked by anti-IL-2R antibody.

IFN-γ-induced IL-2R expression was not restricted to malignant human monocytic cells. By one color (Table III) and two color immunofluorescence (Fig. 2) normal peripheral blood monocyte preparations (99% pure as estimated by morphology and Mo2 staining) could be induced to express IL-2R by recombinant IFN-γ (99.4% of cells stained by biotinylated Mo2 antibody, 62.5% double stained by biotinylated Mo2 and fluoresceinated anti-IL-2R1).

These findings reveal a previously unknown function of IFN-γ. The biological consequences of IL-2R expression by normal monocytes have not been determined, but these results implicate the IL-2/IL-2R system in the control of macrophage differentiation and/or function. It is also possible that macrophage IL-2R expression adsorbs IL-2 at inflammatory sites, and thus serves as a negative feedback loop in T cell/macrophage interaction.

Summary

Gamma interferon induced surface expression of interleukin 2 (IL-2) receptors on normal human monocytes and the monocytoid cell lines U937 and HL60. These receptors were detected by anti-IL-2 receptor monoclonal antibodies, and U937 IL-2 receptors were indistinguishable from T lymphocyte IL-2 receptors by immunoprecipitation. Also, U937 IL-2 receptors bound biologically active IL-2. These results suggest a role for monocyte IL-2 receptors in T cell/monocyte interaction during an immune response.

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