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Human IFN-γ up-regulates IL-2 receptors in mitogen-activated T lymphocytes

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SUMMARY

This study examined the role of human recombinant interferon-gamma (rIFN-γ) in the expression of interleukin-2 receptors (IL-2R) by human T lymphocytes. rIFN-γ enhanced total numbers of IL-2R in mitogen-activated but not resting T cells. Scatchard plot analysis indicated that rIFN-γ increased both high- and low-affinity receptors, with a predominant effect on the latter. Phytohaemagglutinin (PHA)-activated T cells treated with IFN-γ showed higher IL-2 binding and greater IL-2 internalization and degradation than cells treated with PHA alone. There was a corresponding increase of mitogen-driven proliferative responses, indicating an increase of functional receptors in IFN-treated cultures. IFN-γ may influence T-cell activation and proliferation by enhancing expression of IL-2R and promoting IL-2 uptake by mitogen-activated lymphocytes.

INTRODUCTION

IFN-γ is a lymphokine with multiple effects on the immune response (Trinchieri & Perussia, 1985). Most of the immunomodulatory effects of IFN-γ depend on its ability to induce synthesis of proteins and cell-surface markers. For example, IFN-γ enhances expression of HLA class II molecules (Virelizier *et al.*, 1984; Sztein *et al.*, 1984) and high-affinity receptors for immunoglobulin G (IgG) in myelo-monocytic cells (Guyre, Morganelly & Miller, 1983). Recent evidence suggests that it can also modulate expression of IL-2R on leukaemic (Herrman *et al.*, 1985) and monocytic cells (Holter *et al.*, 1986). Since IL-2R are fundamental to the process of T-cell activation (Greene & Leonard, 1986), the potential role of IFN-γ in this cell subpopulation is of critical importance. A previous report by Johnson & Farrar (1983) suggested that an IFN-γ-like cytokine could regulate IL-2R on T cells. The availability of highly purified recombinant IFN-γ has allowed direct examination of this important issue and differentiation of IFN-γ-mediated effects from those of potential contaminants. This report examines the effect of this cytokine on IL-2R expression by resting and activated human T cells.

MATERIALS AND METHODS

Cells

Peripheral blood mononuclear cells (PBMC) from healthy donors were obtained by Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) gradients. T cells were isolated by nylon-wool techniques and further purified by discontinuous Percoll gradients (Rodriguez *et al.*, 1987). Preparations contained 95 ± 1% CD3 (+) cells and less than 0·5% monocytes, as assessed by indirect immunofluorescence with specific monoclonal antibodies (mAb) (Coulter Immunology, Hieleah, FL). Complete culture medium was prepared with RPMI-1640, fetal calf serum (FCS) 10%, L-glutamine and antibiotics (Gibco, Grand Island, NY).

Reagents

rIFN-γ was kindly provided by Dr G. Adolf (Ernst Boehringer, Vienna, Austria). Endotoxin contamination was less than 0·125 ng/mg protein, as tested by the Limulus assay. Anti-CD3 (T3) and anti-monocyte (Mo1) mAb were purchased from Coulter Immunology. Anti-Tac mAb specific for the p55 protein of the IL-2R (Uchiyama, Broder & Waldman, 1981) was a generous gift from Dr T. Waldmann (National Institutes of Health, Bethesda, MD). Other reagents included recombinant IL-2 (rIL-2) (Genzyme Corp., Boston, MA), pronase (protease type XXI from *Streptomyces griseus*; Sigma, St Louis, MO), pokeweed mitogen (PWM; Gibco Laboratories), PHA (Wellcome Research, Beckenham, Kent, U.K.) and [^{3}H]thymidine, specific activity 2 mCi/mM (New England Nuclear, Boston, MA).

Proliferative T-cell responses

T cells (1×10^5) in complete medium were cultured in triplicate in round-bottomed microculture plates and added with PHA (1 μg/ml) and various concentrations of rIFN-γ, or with either reagent alone. Cells were cultured during 72 hr and pulsed with 1 μCi[^{3}H]thymidine 12 hr before terminating the culture. Cells were harvested in a MASH II cell harvester and radioactivity counted in a Rackbeta counter (LKB Wallac, Turku, Finland).

Abbreviations: IFN, interferon; IL-2, interleukin-2; IL-2R, interleukin-2 receptors; PHA, phytohaemagglutinin; TCA, trichloroacetic acid.

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IL-2 binding assay
rIL-2 was iodinated by the chloramine T method (Sugamura *et al.*, 1984). Specific activity of ^{125}I-labelled IL-2 (^{125}I-IL-2) was 0·25–0·3 μCi/pmol. Binding of radioiodinated IL-2 to T cells was measured using methods described by Fujii *et al.* (1986b) with some modifications. T cells (2×10^6) were cultured with PHA (1 μg/ml) or PHA (1 μg/ml) plus rIFN-γ (100 U/ml) for 72 hr. Cells were assayed for viability by trypan blue dye exclusion (>95%), washed and incubated for 1 hr in RPMI-1640 medium containing 20% FCS. Cells were then diluted to 2×10^5 per 100 μl in microtubes and mixed with 100 μl of ^{125}I-IL-2 serially diluted in RPMI containing 0·02% sodium azide. After shaking for 30 min and incubating for 1 hr at 4°, the supernatant (A) was harvested and the cell pellet gently resuspended in 100 μl PBS. The cell suspension was then layered on 750 μl RPMI-1640 medium containing 1 M sucrose and 0·02% bovine serum albumin (BSA) in a microtube. After centrifugation at 8000 ***g*** for 3 min, the supernatant (B) and cell precipitate were harvested. The radioactivities of supernatants (A+B) and cell precipitates were counted; non-specific and specific binding were 16 ± 2 and 83 ± 2 of total binding, respectively, as assessed in the presence of increasing concentrations of unlabelled rIL-2 (>2 μM).

Radioiodinated IL-2 retained its biological activity as shown by the similar supportive effect of unlabelled or ^{125}I-rIL-2 on the growth of PHA-induced T-cell blasts, as checked by the trypan blue dye exclusion under inverted phase microscopy (Table 1; Robb, Kutny & Chowdry, 1983). These results confirm the feasibility of obtaining biologically active IL-2 after iodination by the chloramine T method (Robb, Mayer & Garlick, 1985).

Pronase treatment of T lymphocytes
5×10^6 T cells were treated with 0·025 mg/ml pronase (specific activity 6·5 U/mg) for 15 min at 37° as described by Robb & Rusk (1986). Cells were then centrifuged twice and resuspended in chilled RPMI-1640 with FCS 50% to stop the reaction. Subsequently, cells were washed four times with RPMI-1640, checked for viability by the trypan blue dye stain (>90% viable cells) and resuspended in fresh RPMI medium containing 20% FCS.

Table 1. ^{125}I-rIL-2 retains its biological activity on PHA-induced T-cell blasts*

	Time (hr)			Mean no.
	18	24	48	cells ± SEM
Medium	0·090 (78)	0·073 (64)	0·070 (33)	0·077 ± 0·01
rIL-2	0·172 (80)	0·167 (72)	0·090 (44)	0·143 ± 0·02†
^{125}I-rIL-2	0·150 (76)	0·186 (69)	0·120 (41)	0·152 ± 0·01†

* T-cell blasts were induced with PHA during 72 hr culture. After several washes, cells were added with medium, cold or radioiodinated rIL-2 and the number ($\times10^5$) and percentages (shown in parentheses) of viable cells were examined after the indicated time periods. Viability was tested by the trypan blue dye exclusion.

† $P<0·05$ comparing with cells cultured in medium alone (Student's *t*-test).

Acid treatment of cell-bound IL-2
The amount of surface-bound IL-2 removable by acidic buffer treatment was measured according to the protocol by Fujii *et al.* (1986b). 1×10^6 activated with PHA or co-stimulated with PHA plus rIFN-γ were incubated with 100 pM ^{125}I-IL-2 in RPMI-1640 at 4°, and centrifuged at 12,000 r.p.m. for 3 min. After centrifugation, the radioactivity in the acid removable fractions (supernatants) and in the precipitate was measured.

Measurement of internalization and degradation of IL-2
1×10^6 T cells activated with PHA or PHA plus rIFN-γ were incubated with ^{125}I-rIL-2 and washed as described above. After the last wash with PBS/BSA buffer, cells were resuspended in 1 ml RPMI-1640 and incubated for 2 hr at 25°. Cells were then centrifuged and resuspended at 4° in 1 ml 0·2 M glycine buffer, pH 2·8, for 10 min. Radioactivity in supernatant and precipitate fractions were each counted and subsequently treated overnight at 4° with 1 ml 10% trichloroacetic acid (TCA) in the presence of 1 mg/ml BSA. The TCA-soluble and -insoluble fractions were counted separately. The soluble fraction represents internalized and degraded IL-2 (Fujii *et al.*, 1986b).

Statistical analysis
Statistical analysis was performed by the Student's *t*-test for paired samples.

RESULTS

Effect of rIFN-γ on IL-2R expression by mitogen-activated lymphocytes

Binding studies with ^{125}I-rIL-2 in T lymphocytes activated with PHA are shown in Fig. 1. Scatchard analysis was curvilinear, suggesting two types of receptors: high-affinity ($K_d=6·0\pm1·2$ pM) and low affinity ($K_d=126\pm25$ pM) receptors. In co-stimulated cultures, total numbers of IL-2R per cell were significantly enhanced, with a predominant effect on the lower

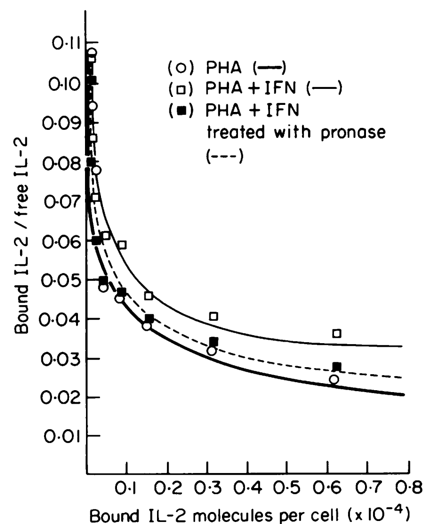

Figure 1. Scatchard analysis of binding experiments using ^{125}I-rIL-2 in T lymphocytes cultured for 72 hr in the presence of PHA alone (1 μg), PHA plus rIFN-γ or PHA plus rIFN-γ in cultures of T cells pre-treated with pronase. Results represent mean ± SD of four experiments.

Table 2. Effect of rIFN-γ in the affinity and number of IL-2R expressed on PHA-activated T cells*

		IL-2R			
		High affinity		Low affinity	
rIFN-γ	Pronase	K_d (pM)	Sites/cell	K_d (pM)	Sites/cell
−	−	6·0±1·2	2380±476	126±25	23,600±720
+	−	9·5±1·9	3500±700†	185±37	54,000±10,800‡
−	+	6·4±0·6	2210±200	95±10	17,000±1710
+	+	7·08±0·07	2900±290	101±11	20,620±2600

* 2 × 10^{6} T-cells were cultured for 72 hr with PHA (1 μg) or PHA plus rIFN-γ (100 U) and the number of IL-2R was determined as described in the Materials and Methods. In some cultures cells were treated with pronase 0·025 mg/ml for 15 min at 37°. Results expressed mean ± SD of four separate experiments.

† $P<0{\cdot}01$ and ‡ $P<0{\cdot}005$ comparing cultures with PHA alone and cultures with PHA plus rIFN-γ.

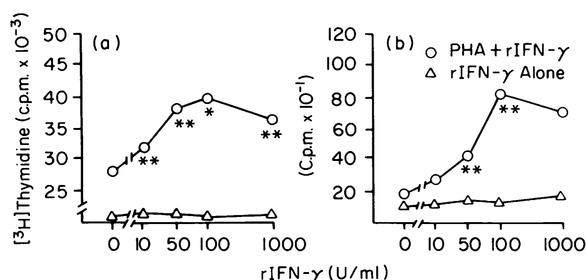

Figure 2. T cells, 1 × 10^{6}, were cultured during 72 hr in round-bottomed microculture plates and stimulated with PHA 0·1 μg (a) or 1 μg (b), rIFN-γ (0–1000 U) or a combination of PHA plus rIFN-γ. Results express means of proliferative responses from four different experiments. $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$.

affinity type ($P<0{\cdot}005$, Table 2). High-affinity receptors were also increased in the presence of rIFN-γ ($P<0{\cdot}01$). No effect was observed in cultures containing rIFN-γ alone in the absence of mitogen (data not shown). Treatment with pronase decreased baseline numbers of low-affinity but not high-affinity IL-2R in cultures activated with PHA alone. Also, this manoeuvre diminished the number and K_d of low-affinity receptors enhanced by rIFN-γ without altering the number of high-affinity receptors (Table 2).

rIFN-γ enhances IL-2 binding and internalization by mitogen-activated T lymphocytes

The percentage of the acid-removable IL-2 fraction was higher in co-stimulated cultures compared with cultures activated with mitogen alone (73·2±4·0 in co-stimulated cultures versus 59·0±4·5 in mitogen alone cultures, $P<0{\cdot}005$, mean±SD, $n=4$). These findings indicated greater amounts of surface-bound IL-2 in T-cell cultures containing rIFN-γ. Moreover, the percentage of degraded IL-2 (supernatant of TCA fraction) was also significantly higher in cultures containing PHA plus rIFN-γ (86±2 in co-stimulated cultures versus 68±7 in mitogen-alone cultures, $P<0{\cdot}05$, mean±SD, $n=3$), indicating greater amounts of internalized IL-2 in co-stimulated cultures than in those activated with mitogen alone.

rIFN-γ enhances proliferative responses by PHA-activated T lymphocytes

rIFN-γ induced a significant enhancement of proliferative responses in PHA-activated cultures at optimal (Fig. 2b) or suboptimal (Fig. 2a) concentrations of mitogen. rIFN-γ alone did not alter baseline proliferation in unstimulated cultures.

DISCUSSION

In the present study, we have examined the role of rIFN-γ in IL-2R expression by T lymphocytes. rIFN-γ enhanced the total number of IL-2R in mitogen-activated but not resting T cells. Also, rIFN-γ augmented the proportion of surface-bound and internalized IL-2R in T lymphocytes activated with mitogen. The enhancement of PHA-driven proliferative responses by T cells co-stimulated with rIFN-γ indicate that this cytokine increases functional IL-2R.

Recent studies have shown that rIFN-γ can up-regulate IL-2R in monocytic cell lines (Herrmann *et al.*, 1985; Holter *et al.*, 1986). Our experiments indicate that IFN-γ can also amplify IL-2R expression in T lymphocytes after an activating signal is delivered. Scatchard plot analysis indicated that both high- and low-affinity IL-2R were up-regulated in co-stimulated cultures, but the effect was stronger on the low-affinity type (Table 2). After pronase treatment (a manoeuvre that selectively depletes T cells from low affinity IL-2R; Robb & Rusk, 1986), IFN-γ lost its ability to increase these receptors over the level expressed by T lymphocytes activated with mitogen alone.

Only high-affinity IL-2R can be internalized after ligand binding (Greene & Leonard 1986; Fujii *et al.*, 1986b; Weissman *et al.*, 1986) and only internalized receptors are capable of transducing signals necessary for T-cell activation (Greene & Leonard, 1986). Our results showed that cultures co-stimulated with PHA plus rIFN-γ had more acid-removable IL-2, indicating greater amounts of surface-bound IL-2 in cultures containing rIFN-γ. Also, the degree of IL-2 internalization was augmented, as demonstrated by higher levels of degraded radioligand in cultures containing IFN-γ. It is possible that, by increasing the number of p55 (Tac) molecules on T cells, IFN-γ may increase the efficiency of IL-2 internalization by high-affinity IL-2R. In favour of this hypothesis, Saito *et al.* (1988) have recently shown stronger association of IL-2 to high-affinity receptors when the number of p55 molecules increases. Alternatively, IFN-γ may render some low-affinity IL-2R amenable to internalization and potentially capable of directly contributing to T-cell proliferation. The net result would be a synergistic effect of IFN-γ and mitogen for T-cell activation and proliferation, as confirmed by experiments shown in Fig. 2.

The possibility that IFN-γ may simultaneously influence the expression of other proteins relevant to the T-cell activation process cannot be ruled out. In fact, HLA class II molecules (Virelizier *et al.*, 1984; Steinz *et al.*, 1984) and IgG Fc-receptors (Guyre *et al.*, 1983) present at low density in resting T cells are increased by IFN-γ in cells of myelo-monocytic origin.

Recent studies have shown that the binding patterns of IL-2 ɔ IL-2R can be modulated by proteolytic treatment (Robb & ƚusk, 1986) or exposure to specific mAb (Fujii *et al.*, 1986a). 'hus, it is possible that IFN-γ may be one of several lymphocyte ıroducts capable of influencing the formation and internaliza-ion of IL-2/IL-2R complexes and potentiate signals needed to arry T cells along the activation and proliferation pathways.

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