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Demonstration and Partial Characterization of the Interferon-Gamma Receptor on Human B Lymphocytes

Toshimasa Nakagawa, Naoko Nakagawa, Gita A. Delsing, David Volkman, and John H. Kehrl

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

The expression of interferon-γ (IFN-γ) receptors on normal human B cells and four B cell lines was studied. Recombinant human IFN-γ was labeled with [γ-^{32}P]ATP using the catalytic subunit of a cAMP-dependent protein kinase. All four B cell lines, although differing in their responsiveness to IFN-γ, were found to express high-affinity receptors (1,000–11,000 receptors/cell). Normal unactivated B lymphocytes were also found to express constitutively high-affinity receptors, approximately 1,400 receptors per cell with an estimated affinity of 295 pM. Activation of the normal B cells in vitro with the polyclonal B cell activator, *Staphylococcus aureus* Cowan strain I (SAC), resulted in a slight decline in receptor number and a more pronounced fall in receptor density. One of the B cell lines and unactivated normal B cells were shown to internalize labeled IFN-γ rapidly. Chemical cross-linking of ^{32}P-IFN-γ to the CB B cell line and to freshly isolated B lymphocytes revealed one major cross-linked receptor-ligand complex which had an estimated molecular weight of approximately 110 kilodaltons. This complex corresponded to a 93 kD receptor cross-linked to recombinant IFN-γ. Our data indicate that normal B lymphocytes constitutively express an approximately 93 kD IFN-γ receptor which is similar to the receptor present on Epstein-Barr virus-transformed B cell lines.

Key words: γ-interferon, receptors, B lymphocytes, internalization

The interferons (IFN) are a family of proteins (α, β, and γ) classically defined by their antiviral activity [1]. IFN-γ is a product of activated lymphocytes, is structurally and functionally different from IFN-α and IFN-β, and is known to bind to a distinct receptor [2–10]. In addition to its antiviral activity, IFN-γ modulates a variety of immunological processes including in vitro B lymphocyte function.

Toshimasa Nakagawa and Naoko Nakagawa are now at Transgene S.A. 11 Rue de Molsheim, 67000 Strasbourg, France.

David Volkman is now at Department of Medicine, State University of New York Health Sciences Center, T-16, room 040, Stony Brook, NY 11794.

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Models of mature B cell function propose that B cells reside in distinct states—resting, activated, proliferating, and immunoglobulin (Ig) secreting. Transitions between these states are thought to be controlled by a variety of signals, including soluble factors secreted by T cells [11–13]. Functional studies of IFN-γ effects on human B cells have demonstrated a potential role for it in regulating B cells in each of these phases. For example, the preincubation of resting B cells with IFN-γ for 24 h enhances their subsequent proliferative response to anti-Ig stimulation [14]. Furthermore, although IFN-γ is traditionally considered to be an antiproliferative factor, it has been shown to costimulate with anti-μ in the induction of B cell proliferation and to maintain weakly the proliferation of B cells previously stimulated by *Staphylococcus aureus* Cowan strain I (SAC). Thus, an effect of IFN-γ has been demonstrated on both activated and proliferating B cells [15,16]. Finally, the addition of IFN-γ to interleukin-2 (IL-2) has been shown to enhance significantly Ig secretion by both SAC-activated B cells and an Epstein-Barr virus (EBV)-transformed B cell line when compared to the enhancement with IL-2 alone [17–19]. IFN-γ has also been shown to modulate murine B cell function, including the regulation of Ig isotype production [20–22].

These findings of multiple effects of IFN-γ on human B cell function led us to examine the expression of IFN-γ receptors on normal B cells and various B cell lines, including a cell line previously shown to be responsive to IFN-γ. Additionally, we have partially characterized the receptor on normal B cells and B cell lines by ligand-receptor cross-linking studies.

MATERIALS AND METHODS

Chemicals and Reagents

Dithiothreitol was purchased from Calbiochem-Behring (La Jolla, CA). Acrylamide, N,N′-methylene-bis-acrylamide, and sodium dodecyl sulfate (SDS) were obtained from Bio-Rad (Richmond, CA). [γ-^{32}P]ATP with a specific activity of 5,000 Ci/mmol (185 TBq/mmol) was purchased from Amersham Co. (Arlington Heights, IL). The catalytic subunit of cyclic AMP-dependent protein kinase from bovine heart muscle with a specific activity of 20,000 U/ml was obtained from Sigma Chemical Co. (St. Louis, MO). SAC was purchased from Bethesda Research laboratories (Gaithersburg, MD), and disuccinimidyl suberate (DSS) was obtained from Pierce Chemical Co. (Rockford, IL). Ficoll-Paque and Sephadex G-10 were purchased from Pharmacia (Piscataway, NJ).

Cell Lines

The EBV-immortalized cells lines—CB, CESS, and SKW6.4—were maintained in RPMI 1640 with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY) at a cell density of 1×10^5 to 1×10^6/ml. CB was established in this laboratory from a normal individual as described [23]. It expresses Tac-Antigen (30–40%), surface IgG (30–60%), and Epstein-Barr virus (EBV) nuclear antigen, but does not express p19 (human T cell leukemia virus-1-associated surface antigen) [24]. CB differentiates in response to IL-2 and IFN-γ synergistically [18]. CESS and SKW6.4 are cell lines which are able to differentiate in response to B cell stimulatory factor-2 (BSF_2) [25,26]. The human Burkitt lymphoma cell line Raji and U937 which was originally established from a patient with histiocytic lymphoma, were maintained as above.

Normal Human B and T Cell Preparation

Venous blood was obtained from normal volunteers, and mononuclear cells (MNC) were isolated by the standard Ficoll-Paque density gradient centrifugation method. 2-Aminoethylisothiouronium bromide-treated sheep red blood cells (AET-SRBC) rosette-negative cells were separated from MNC by Ficoll-Paque centrifugation. Macrophages were depleted by passage over Sephadex G-10 columns. These B cell preparations were 72% ± 9% sIg^+, consistently less than 2% esterase positive, and less than 1% OKT3 positive. To obtain SAC-activated B cells, these B cells were cultured with SAC (1:25,000) for 3 or 5 days, and viable cells were obtained by Ficoll-Paque centrifugation. Tonsillar B cells were prepared as previously described [27]. The tonsillar B cells were greater than 95% sIg^+, less than 1% $OKT3^+$, and less than 1% esterase positive. AET positive cells were used as T cells and activated with phytohemagglutinin for 2 days.

Labeling of Human IFN-γ With [γ-^{32}P]ATP

Recombinant human IFN-γ was a generous gift from Genentech Inc. (South San Francisco, CA). The phosphorylation of IFN-γ was performed according to the method described by Rashidbaigi et al. [28]. In brief, 2 μg of IFN-γ, were incubated at 37°C for 15 min with 1 mCi of [γ-^{32}P]ATP and 10 U of the catalytic subunit of cAMP-dependent protein kinase from bovine heart muscle in 30 μl of 20 mM Tris HCl (pH 7.4), 1 mM dithiothreitol, 100 mM NaCl, and 12 mM $MgCl_2$. The reaction was stopped by the addition of 1.0 ml of 1 mg/ml bovine serum albumin (BSA) (Sigma) in a buffer containing 10 mM EDTA, 10 mM sodium phosphate, 10 mM sodium pyrophosphate, pH 7.0 (PPE). The ^{32}P-labeled IFN-γ was dialyzed against 3 liters of PPE overnight at 4°C, and then against 1 liter of PPE for 4 h. The specific activity of 247.7 μCi/μg was calculated based on an estimated 10% loss of IFN-γ during dialysis. [^{32}P]IFN-γ was apportioned into 100 μl fractions, frozen, and stored at −70°C. SDS-PAGE of [^{32}P]IFN-γ was performed on a 15% slab gel by the method of Laemmli [29].

Binding Assay of [^{32}P]IFN-γ

The IFN-γ binding assay was performed according to the methods described by Rashidbaighi et al. [28], and Robb et al. [30], although slightly modified. A total of 1×10^6 cells were preincubated in the presence or absence of 5×10^{-8} M unlabeled IFN-γ for 30 min at 4°C in a total volume of 100 μl of medium (RPMI 1640 supplemented with 10% FCS) in 1.5 ml of Eppendorf microtest tubes (Brinkman Instrument, Westbury, NY). After preincubation, serial dilution of [^{32}P]IFN-γ were added to the cells and they were incubated at 4°C for 120 min. Subsequently, 1 ml of ice-cold medium was added to each tube, and the cells were spun down at 10,000*g* for 30 sec in an Eppendorf model 5414 centrifuge (Brinkman instrument). The cell pellet was resuspended in 100 μl of medium and centrifuged at 10,000*g* for 90 sec through a 200 μl layer of mixture of 84% silicon oil and 16% paraffin oil (Fischer Scientific Co., Philadelphia, PA) in a 0.4 ml polyethylene tube (Bio-Rad). The tips of the tubes containing the cell pellet were cut off, resuspended in 100 μl of 1% SDS, and counted in a scintillation counter. Specific binding was calculated by subtracting the amount of nonspecific binding. The affinity and number of binding sites were calculated by Scatchard analysis of the binding curves [31]. The molecular weight of monomeric IFN-γ was used in the calculations.

Internalization of Surface-Bound [^{32}P]-IFN-γ

The internalization procedure was performed according to methods described by Anderson et al. [32] and Klausner et al. [33], with slight modification. Cells (1×10^7/ml) were incubated in the presence or absence of 5×10^{-8} M unlabeled IFN-γ for 30 min at 4°C in RPMI 1640-BSA (25 mM Hepes, pH 7.2, 5 mg/ml BSA). [^{32}P]IFN-γ was added to the cells at a final concentration of 1×10^{-10}M, and after a 2 h incubation at 4°C, the cells were washed with ice-cold RPMI 1640-BSA. The cells were resuspended with prewarmed RPMI 1640-BSA containing chloroquine at 200 μM, and incubated at 37°C. After various lengths of incubation (0–60 min), 1×10^6 cells (100 μl) were removed from the culture and added to 100 μl of 0.25 M acetic acid/0.5 M NaCl (pH 2.3). After 5 sec, 50 μM of 1 M sodium acetate was added, to return the pH to 6.0, and the cells were immediately spun through the silicon/paraffin mixture. Cell recovery was unaffected by the exposure to acid, and the incubation with the acid for longer times did not alter the amount of ligand released. In parallel 150 μl RPMI 1640 was added to the cells instead of acid treatment and the cells spun through the silicon/parafin mixture. Data shown in Table II were calculated by subtracting nonspecific binding (cpm in the presence of cold IFN-γ) from each of the samples.

Cross-Linking of [^{32}P]IFN-γ to the Cell Membrane and SDS-PAGE Analysis

A total of 2.0×10^7 cells (CB cells or normal tonsil B cells) were washed with ice-cold RPMI 1640 supplemented with 2% FCS, 25 mM Hepes, pH 7.4 (binding medium). Then the cells were incubated in 1 ml of this media with 500 pM [^{32}P]IFN-γ for 2 hr at 4°C on a rotor. In parallel, control cells were incubated with a 100-fold excess of unlabeled IFN-γ. Following the incubation, the cells were washed with cold media, resuspended in 1 ml of cross-link buffer (PBS, 1 mM $MgCl_2$, 0.02% NaN_3 pH 8.3), and 10 μl of 10 mg/ml DSS in dimethylsulfoxide (DMSO) was added. The cells were rotated for 30 min at room temperature and then washed 3 times with cold PBS. The cross-linked cells were lysed with a 1% Nonidet P-40 (NP40) buffer containing 150 mM NaCl, 10 mM Tris pH 7.4, and 1 mM phenylmethylsultonylfluoride (PMSF). The cell lysates were immunoprecipitated with a polyclonal anti-IFN-γ (Interferon Sciences, New Brunswick, NJ) antibody coupled to protein A-Sepharose. The immunoprecipitates were washed twice with the lysis buffer resuspended in SDS sample buffer, boiled for 5 min, and subjected to SDS-PAGE [29].

RESULTS

Characterization of [^{32}P]IFN-γ and Its Binding to U937 Cells

Recombinant IFN-γ was phosphorylated with [γ-^{32}P]ATP and a bovine heart protein kinase according to the method described by Rashidbaigi et al. [28]. This method allows the preparation of radiolabeled interferon of very high specific activity yet without loss of biologic activity. Analysis of the phosphorylated interferon by SDS-PAGE and autoradiography revealed a single band with an estimated molecular size of 17 kD (Fig. 1). Longer exposure revealed a band at 34 kD corresponding to the dimeric form of IFN (data not shown).

Next control experiments were performed with U937 cells, a cell line previously used in IFN binding studies. U937 cells were incubated with increasing concentrations

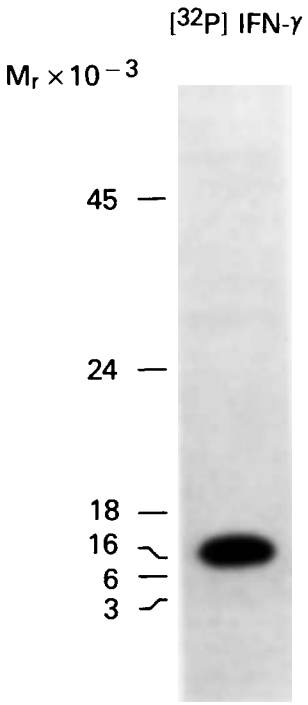

Fig. 1. SDS-PAGE of [^{32}P]IFN-γ. Human recombinant IFN-γ was labeled with ^{32}P and analyzed by SDS-PAGE as described in Materials and Methods.

of [^{32}P]IFN-γ varying from 6×10^{-12} M to 6×10^{-9} M. Nonspecific binding was determined in the presence of 5×10^{-8} M unlabeled IFN-γ. Scatchard analysis of the binding data revealed 2,200 binding sites per cell with an estimated affinity of 173 pM (Fig. 2). These results are in agreement with binding data from U937 cells reported by Rashidbaigi et al. (1,800 sites per cell with a kd of 150 pM) [28]. In order to assess the binding ability of the [^{32}]IFN-γ, an increasing number of U937 were incubated with a constant amount of [^{32}P]IFN-γ (8×10^{-11}M). Specific binding increased with increasing cell number until saturation occurred, at which point 80% of the added [^{32}P]-IFN-γ was observed to be bound to the cells (data not shown).

Equilibrium Binding of [^{32}P]IFN-γ to Human B Cell Lines and Normal B Cells

Four B cell lines—CB, CESS, SKW6.4, and Raji—were examined for the expression of IFN-γ receptors. The CESS and SKW6.4 B cell lines have been shown to respond to a B cell differentiation factor, BSF2, by increased Ig secretion [25,26]. The CESS cell line is unresponsive to either IL-2, IFN-γ, or the combination, while the SKW6.4 cell line responds to IL-2, but only when IL-2 is present at high concentrations [17,34]. The CB cell line expresses low levels of the Tac antigen, one component of the IL-2 receptor, and responds to IL-2 with increased Ig secretion which can be further augmented by the addition of IFN-γ [18]. Finally the Raji cell line is unresponsive to IL-2, IFN-γ, and BSF2 (J. Kehrl, unpublished observation). Scatchard analysis of the binding data obtained with CESS and CB cells is shown in Figure 3 and the results of the

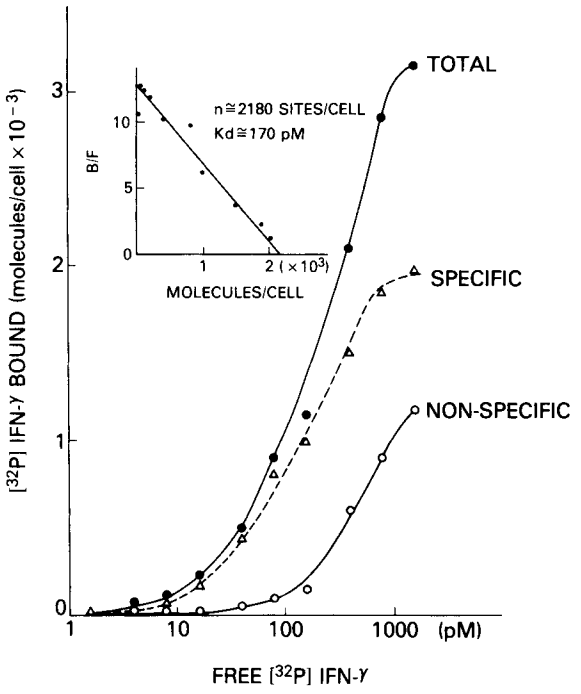

Fig. 2. Equilibrium binding of [^{32}P]IFN-γ to U937 cells. The binding assay was performed as outlined in Materials and Methods. The broken line (open triangles) is the specific binding calculated by subtracting nonspecific binding (open circles) from total binding (closed circles). Each points represents the mean of duplicate determination. **Inset:** Scatchard analysis of the binding data.

Scatchard analysis of the binding data with SKW6.4 cells and Raji cell are presented in Table I. CESS and SKW6.4 have approximately 1,000 receptors per cell while Raji cells and CB cells have tenfold more receptors. All the cell lines have similar affinities for the radiolabeled interferon, although somewhat surprisingly, the CB cell line has the lowest affinity of the four cell lines examined.

We next examined the expression of IFN-γ receptors on normal peripheral blood B cells. Previous functional studies had shown an effect of IFN-γ on unactivated B cells, suggesting that the receptor should be constitutively expressed on them [14]. A [^{32}P]-IFN-γ binding assay demonstrated the presence of IFN-γ receptors on purified peripheral blood B cells, and Scatchard analysis of the data revealed a similar number of receptors per cell and estimated affinity as was observed with SKW6.4 and CESS cells. Since the density of many receptors is increased following B cell activation, the effects of SAC-activation upon receptor levels was examined. Following 3 and 5 days of culture in the presence of SAC, the B cells were harvested and [^{32}P]-IFN-γ binding assays were performed (Fig. 4; Table I). Activation with SAC did not increase the number of receptors present. Rather, there was a slight reduction in receptor numbers per cell, and since the cells enlarge following activation, a more pronounced fall in receptor density. These experiments were performed three times with consistent results. We also observed a decline in IFN-γ receptor density following T cell activation (Table I). These latter results are in agreement with previously reported observations [35].

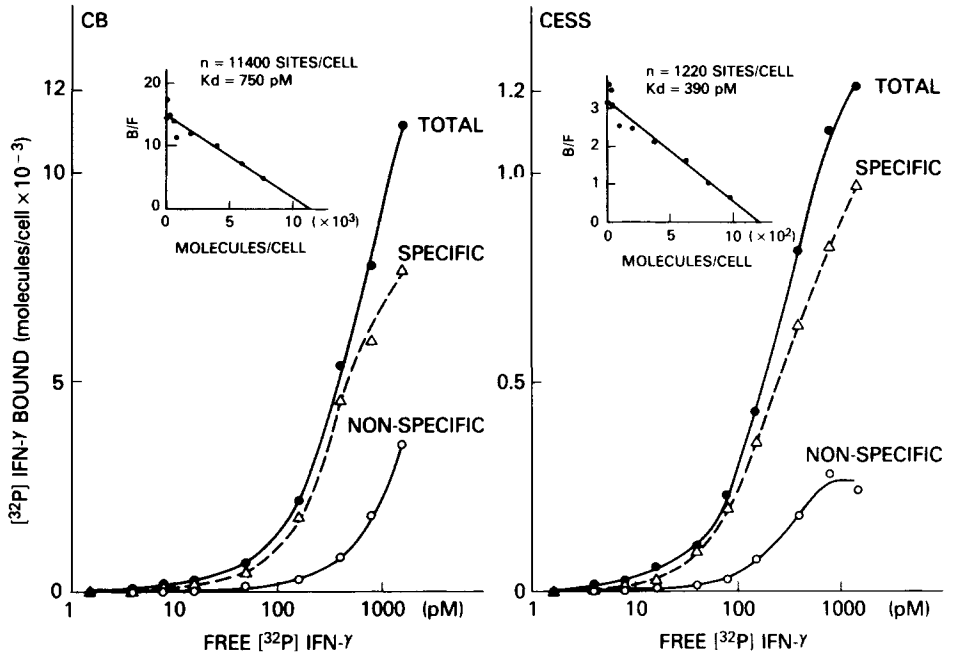

Fig. 3. Equilibrium binding of [^{32}P]IFN-γ to CB and CESS cells. The binding assay was performed as outlined in Materials and Methods. The broken line (open triangles) is the specific binding calculated by subtracting nonspecific binding (open circles) from total binding (closed circles). **Inset:** Scatchard analysis of the binding data.

Internalization of Surface Bound IFN-γ

Internalization and degradation of IFN-γ was first demonstrated with trisomic fibroblasts [2]. We studied the internalization of IFN-γ by the interferon responsive B cell line, CB. Cells were incubated with a 1×10^{-10} M concentration of [^{32}P]-IFN-γ in the presence or the absence of unlabeled IFN-γ at 4°C. The cells were resuspended in 37°C medium and surface-bound labeled IFN-γ was removed at various time points by a brief exposure to pH 2.3 media and the remaining cell associated [^{32}P]-IFN-γ was then determined (Table II). Approximately 80% of the cell-associated radioactivity was

TABLE I. Summary of Binding Assays

Cells	K_d(pM)	Receptors/cell
U937	173	2,180
CB	750	11,400
CESS	393	1,220
SKW6.4	423	1,100
RAJI	160	11,700
Resting B	295	1,360
3D-SAC-activated B	330	1,280
5D-SAC-activated B	242	1,020
Resting T	215	630
2D-PHA activated T	240	540

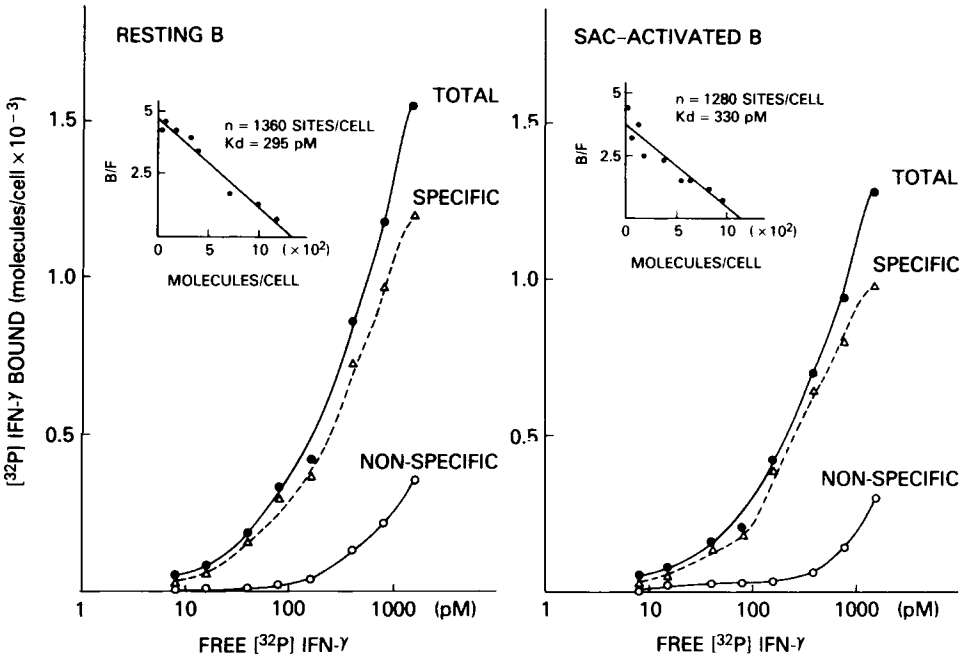

Fig. 4. Equilibrium binding of [^{32}P]IFN-γ to resting and 3 day SAC-activated B cells. The binding assay was performed as outlined in Materials and Methods. The broken line (open triangles) is the specific binding calculated by subtracting nonspecific binding (open circles) from total binding (closed circles). **Inset:** Scatchard analysis of the binding data.

eluted with acid treatment at the beginning of the incubation. Within 5 min there was a significant increase in acid-resistant cell-associated radioactivity, and by 15 min there was no further increase in acid-resistant CPMs. The internalization was done in the presence of chloroquine to prevent dissociation of the IFN-γ receptor complex and its subsequent degradation. Similar results were found with the normal B cells (data not shown).

TABLE II. Internalization of ^{32}P-IFN-γ by CB Cells*

Time (min)	Cell-associated cpm following (cpm/10^6 cells)		% acid-resistant counts
	Acid wash	RPMI wash	
0	1574 ± 41	8149 ± 327	19.0
5	2756 ± 41	8313 ± 132	33.0
10	2743 ± 57	7452 ± 179	36.8
15	3472 ± 14	6653 ± 49	52.1
30	2873 ± 202	5945 ± 26	48.0
60	3142 ± 27	5865 ± 108	53.0

*The procedure to detect the internalization of surface-bound [^{32}P]IFN-γ was performed according to the method outlined in the Materials and Methods. Percentage of acid-resistant counts was calculated by dividing the cell-associated cpm following acid wash by associated cpm following RPMI wash.

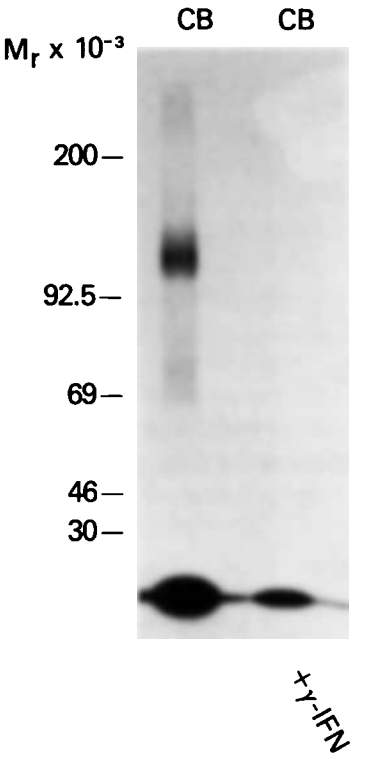

Fig. 5. [^{32}P]-IFN-γ cross-linking to CB cells. 20 × 10^6 CB cells were cross-linked to [^{32}P]-IFN-γ at a final concentration of 500 pM in the presence or absence of a 100-fold molar excess of unlabeled IFN-γ with dissuccinimydyl suberate (DSS). The cross-linked cells were solubilized in a 1% NP40 lysis buffer, immunoprecipitated with a polyclonal anti-IFN-γ antibody, and analyzed on SDS polyacrylamide gels. ^{14}C-labeled proteins were used as molecular weight markers.

Cross-linking of [^{32}P]-IFN-γ to CB Cells and Normal B Cells

In order to characterize the IFN-γ receptor on human B cells, [^{32}P]-IFN-γ was chemically cross-linked to the cell surface membranes of CB cells and normal B cells using the homobifunctional cross-linker disuccinimidyl suberate. Following cross-linking the cells were solubilized in a NP-40 lysis buffer, immunoprecipitated with an anti-IFN-γ antibody, and analyzed by SDS-PAGE. One major receptor IFN-γ complex was identified with CB cells which migrated with an estimated molecular size of 110 kD (Fig. 5). Similar results were obtained when the cross-linked complexes were directly analyzed by SDS-PAGE; however, the resolution of the complexes was improved by immunoprecipitation with the anti-IFN-γ antibody (data not shown). The identified receptor IFN-γ complex corresponded to a protein of approximately 93 kD cross-linked to IFN-γ. Using a similar approach, we examined the IFN-γ binding proteins present on normal B cells. Unactivated or 2 day SAC-activated B cells were cross-linked to [^{32}P]-IFN-γ and immunoprecipitated with the anti-IFN-γ antibody (Fig. 6). Again, one major band was identified. The ligand-receptor complex had an estimated molecular weight of approximately 110 kD, which again corresponded to an 93 kD protein complexed to IFN-γ. A

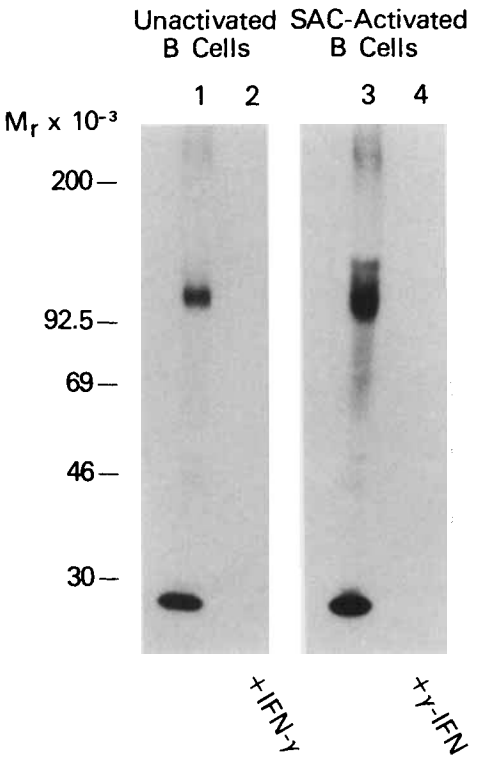

Fig. 6. [^{32}P]-IFN-γ cross-linking to normal B cells. 20 × 10^6 tonsillar B cells or 20 × 10^6 SAC-activated B cells were cross-linked to [^{32}P]-IFN-γ at a final concentration of 500 pM in the presence or absence of a 100-fold molar excess of unlabeled IFN-γ with DSS. The cross-linked cells were solubilized, immunoprecipitated, and analyzed by SDS-PAGE. ^{14}C-labeled molecular weight markers were used as standards.

less prominent band was present at approximately 127 kD, which may represent the 93 kD protein cross-linked to the dimeric form of IFN-γ.

DISCUSSION

In this study we demonstrate the presence of IFN-γ receptors on four B cell lines and normal B lymphocytes isolated from either peripheral blood or tonsils. The four B cell lines differed in their responsiveness to IFN-γ, yet all expressed receptors. Unactivated peripheral blood B cells were found to have approximately 1,400 receptors per cell with an estimated affinity of 295 pM. Activation of the B cells with the polyclonal B cell activator SAC did not significantly modulate the number of receptors per cell nor its affinity for IFN-γ. The CB cells, an IFN-γ-responsive cell line, were shown to internalize [^{32}P]-IFN-γ as were normal B cells. Finally, the receptor was studied on the CB cell line and normal B cells by [^{32}P]-IFN-γ chemical cross-linking. A prominent interferon binding protein of approximately 93 kD was identified on both normal B cells and the CB cell line.

The existence of a specific IFN-γ receptor was first identified by binding experiments with [^{125}I]-IFN-γ using trisomic fibroblasts [2]. Subsequently, receptors have

been identified on a variety of normal and transformed cells, including fibroblasts, epithelial cells, T lymphocytes, NK cells, and various cell lines of hematopoietic origin [3–10]. Although there are some exceptions, Scatchard analysis of the binding data has revealed a single high-affinity binding site. Scatchard analysis of binding data derived from normal human B cells also revealed a single affinity binding site which varied from 250 to 330 pM. Studies with normal T cells revealed approximately 600 receptors per cell with an estimated affinity of 180 pM. Thus, normal B cells appear to have approximately twofold more receptors per cell than T cells, although a similar affinity for IFN-γ. This difference may be important, since the occupancy of only a small percentage of the available receptors appears to be necessary for most biological effects [36]. At low concentrations of ligand, likely to be the case in vivo, a twofold increase in receptors will result in a twofold increase in the occupied receptors per cell, and a twofold increase in cell response. B cell activation is accompanied by the up-regulation of a variety of membrane proteins, including HLA-DR, transferrin, and IL-2 receptors; however, B cell activation had no significant effect on the number or the affinity of the IFN-γ receptor. Since B cells enlarge during activation, there is an actual decline in the density of receptors following in vitro activation.

Other studies have reported IFN-γ binding affinities tenfold lower to tenfold higher than reported here [2–10]. Most of those studies used iodinated rather than phosphorylated IFN-γ. The use of ^{32}P-IFN-γ has certain advantages over iodinated material. For example, the specific activity of the ^{32}P-IFN-γ is 10–100-fold greater than iodinated preparations. Also, the phosphorylation of IFN-γ does not interfere with its biologic activity [28], and the majority of the ^{32}P-IFN-γ can bind to cells (as high as 80% in some experiments). The high specific activity and specificity of binding permits accurate equilibrium binding studies. Our studies with U937 cells are in close agreement with results previously published by Rashidbaigi et al. [28], who also examined the binding of ^{32}P-IFN-γ to U937 cells. While the ^{32}P-IFN-γ was an excellent reagent for binding studies, it may have some disadvantages when used in ligand internalization studies. For example, we found a higher percentage of acid-resistant counts at the initiation of the 37°C incubation (time = 0 min) than is usually found with iodinated interferon. Additionally, the decrease in cell-associated radioactivity during the 37°C incubation may be secondary to dephosphorylation of the ^{32}P-IFN-γ by endogenous phosphatases.

Ucer et al. [37] have recently reported their results analyzing the binding of ^{125}-IFN-γ to normal T and B lymphocytes and several different B cell lines. Their binding studies with Raji cells revealed 3,900 receptors per cell with an estimated affinity of 20 pM. These results differ significantly from our results with Raji cells (11,400 receptors per cell with an estimated affinity of 160 pM). Part of the difference in the calculated number of receptors per cell and estimated affinity is accounted for by our use of the monomeric molecular weight of IFN-γ, while Ucer et al. [37] used the dimeric molecular weight in their calculations. However, if our data are recalculated using the dimeric molecular weight, there remains a significant difference, particularly in estimated receptor affinity. The reasons for this difference are unclear. Our results with Raji cells are in relatively close agreement with those recently reported by Aguet and Merlin [38], who found an estimated binding affinity of IFN-γ for its receptor on Raji cells of 100 pM with approximately 10,000 sites per cell.

The presence of IFN-γ receptors on resting B cells suggests a potential role for IFN-γ in the regulation of B cell activation. Although the addition of IFN-γ to resting B cells has no effect on B cell HLA-DR expression, RNA synthesis, cell size, DNA synthesis, or Ig secretion [39], preincubation of resting B cell with IFN-γ results in a subsequent enhanced proliferative response to anti-Ig mediated signals [14]. This suggests a direct effect of IFN-γ on resting B cells. In other cell systems the effect of IFN-γ on cellular activation has been studied in more detail. For example, exposure of monocytes to IFN-γ results in a slow rise in intracellular Ca^{++} levels and increased activity of protein kinase C [40–41]. Similar events may occur in B cells to account for their increased proliferative response following exposure to IFN-γ.

IFN-γ has also been reported to have BCGF activity in the anti-μ costimulation assay and the SAC assay [15,16]. Additionally, IFN-γ has been shown to enhance B cell differentiation when present with other differentiation signals [17,18]. We have previously described an EBV-transformed B cell line CB whose differentiation to an Ig-secreting cell is stimulated by IL-2 and have shown that this response can be amplified by the addition of IFN-γ [18]. A kinetic study revealed that IFN-γ was required late in culture rather than early [18]. These results in conjunction with the binding data suggest that the different effects of IFN-γ on B cell function are dependent upon the activation or differentiation of the B cell and upon the other cytokines present rather than altered levels of receptors.

Several reports have included analysis of ligand receptor complexes. Rubenstein et al. [35] have identified two molecular forms of the receptor. Monocytes and several lines of hematopoietic origin expressed a receptor of approximately 140 kD while fibroblast expressed a receptor of approximately 95 kD. Ucer et al. [37] identified three ligand receptor complexes on a variety of cell lines, including K562 and a colon carcinoma-derived cell line with estimated molecular weights of 70, 92, and 160 kD. These complexes correspond to three species with estimated molecular weights of 50, 72, and 140 kD, respectively. Whether these represent subunits of the receptor or split products of the receptor remain to be established. More recently, Aguet and Merlin have described the development of an antireceptor monoclonal antibody which has been used in conjunction with affinity chromatography partially to purify the IFN-γ receptor from Raji cells [38]. They have identified two proteins with molecular weights of 90 (p90) and 50 (p50) kD. The p50 protein was the more prominent, with p90 never exceeding 30% of the total [38]. In contrast, our results suggest that the p90 protein is the predominant receptor expressed on CB cells. A band which would correspond to p50 was only visualized when the complex was immunoprecipitated and the autoradiograph overexposed (data not shown). We have also immunoprecipitated [^{32}P]-IFN-γ cross-linked to Raji cells and found similar results as with CB cells (J. Kehrl, unpublished observation). This data suggests that the p50 protein may be a degradation product of the p90 protein that retains the IFN-γ binding site. The 160 kD ligand-receptor complex present on monocytes was not observed with the CB cells.

Analysis of [^{32}P]-IFN-γ cross-linked protein on unactivated normal B cell revealed a band at 110 kD which presumably corresponded to p90. Immunoprecipitation of cross-linked proteins from the activated B cells also revealed a prominent band at approximately 110 kD. A second band at approximately 127 kD was also present on the normal B cell. Although more evident on the SAC-activated B cells, longer exposures

revealed its presence on the unactivated B cells. This band may correspond to dimeric interferon cross-linked to p90.

In conclusion, we have demonstrated the expression of IFN-γ receptor on resting B cell and a reduction in receptor density following B cell activation. Both the CB cell line and normal B cells were found to internalize IFN-γ rapidly. Chemical cross-linking studies with [^{32}P]-IFN-γ revealed a predominant binding protein of 93 kD present on both normal B cells and CB cells. Knowledge of the expression and the modulation of IFN-γ receptors on normal B cells will assist in our understanding the role of IFN-γ in the regulation of human B cell function.

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