

Antagonistic Action of IFN- β and IFN- γ on High Affinity Fc γ Receptor Expression in Healthy Controls and Multiple Sclerosis Patients¹

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Monocyte-macrophage activation by IFN- γ is characterized by a pronounced increase of high affinity Fc receptors for IgG (Fc γ RI), capable of triggering respiratory burst, phagocytosis, Ab-dependent cytotoxicity, and release of proinflammatory cytokines. In view of the antagonism of IFN- β on IFN- γ action, of interest in the chronic inflammatory disorder multiple sclerosis, we examined the possible effect of IFN- β on IFN- γ induction of Fc γ RI gene expression. We found that IFN- β significantly down-regulated IFN- γ -induced Fc γ RI surface expression in peripheral blood monocytes from healthy donors, in a dose- and time-dependent manner. This down-regulation of Fc γ RI surface levels did not correspond to a decrease in Fc γ RI mRNA, suggesting a posttranscriptional effect of IFN- β . Down-regulation of Fc γ RI surface expression correlated with diminished cellular signaling through Fc γ RI, since the IFN- γ -induced increase in Fc γ receptor-triggered respiratory burst was nearly completely abrogated by simultaneous addition of IFN- β . Finally, the same antagonism between both IFNs on Fc γ RI surface expression was observed in peripheral blood monocytes derived from multiple sclerosis patients; inhibition by IFN- β was even increased ($82 \pm 11\%$), as compared with healthy controls ($67 \pm 4\%$). These results may partially help explain the beneficial effect of IFN- β in multiple sclerosis. *The Journal of Immunology*, 1998, 161: 1568–1574.

The antagonism of IFN- β on IFN- γ action has gained considerable clinical significance, in view of their opposite effects in the chronic inflammatory disorder multiple sclerosis (MS)³. Administration of IFN- γ to MS patients has been shown to provoke exacerbations of the disease (1), whereas IFN- β treatment reduced exacerbations and actively diminished disease progression as monitored by magnetic-resonance brain scanning (2-4). This antagonistic action has been extensively documented in vitro for MHC class II gene regulation (5-7), which may partly explain the beneficial effect of IFN- β in vivo, by down-regulating pathogenic IFN- γ -induced MHC class II expression in the central nervous system, where it is absent under normal physiologic conditions (8, 9).

Central nervous system Ag presentation through IFN- γ -induced MHC class II expression leads to activation of autoreactive T cells, a primary event in the pathogenesis of MS and of its animal models (10-12). This was demonstrated by adoptive transfer of the

disease by encephalitogenic T cells in rodents (13, 14) and recently in the *Callithrix jacchus* primate model (15), which more closely resembles MS. However, autoreactive T cells are not sufficient to provoke experimental allergic encephalomyelitis (EAE), since IgG-deficient rats fail to develop EAE (16) and, in *C. jacchus*, encephalitogenic T cells only cause full demyelination in the presence of anti-myelin Abs (17). In MS patients as well, data on cellular (auto)immune reactivity predominate, but humoral immune reactivity to myelin Ags has been demonstrated (18, 19).

Moreover, in later stages of the disease, T lymphocytes are greatly outnumbered by macrophages (20), which in fact execute the final step in demyelination, i.e., myelin phagocytosis (21). Capping of IgG on macrophages in MS plaques, differential uptake of opsonized myelin by macrophages and microglia, macrophage attachment of oligodendrocytes in the presence of anti-myelin Abs, and increased Fc receptor expression in MS lesions (22-25) argue for a deleterious role for Fc receptors in demyelination.

Monocyte-macrophage activation by IFN- γ is characterized by a pronounced increase of high affinity Fc receptors for IgG (Fc γ RI, CD64), capable of mediating phagocytosis, respiratory burst, Ab-dependent cytotoxicity, and secretion of proinflammatory cytokines, such as TNF (26), that have been incriminated in MS and EAE pathogenesis (20, 27-30).

Therefore, we envisaged a possible effect of IFN- β on IFN- γ induction of Fc γ RI, which, besides its likely pathologic significance, was also particularly interesting from the viewpoint of IFN signaling. Elucidation of the process by which IFNs induce transcription of immediate response genes has revealed two families of proteins required for signal transduction by IFNs and numerous other cytokines and growth factors (31). The first protein family consists of receptor-associated Janus protein tyrosine kinases (JAKs); the second comprises a group of latent cytoplasmic transcription factors, designated signal transducers and activators of transcription (STATs), which become activated and translocate

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³ Abbreviations used in this paper: MS, multiple sclerosis; CIITA, class II *trans*-activator; EAE, experimental allergic encephalomyelitis; Fc γ RI, high affinity Fc receptor for IgG; GAS, γ -IFN-activated sequence; MFI, mean fluorescence intensity; JAK, Janus protein tyrosine kinase.

to the nucleus upon tyrosine phosphorylation (31, 32). Fc γ RI mRNA induction by IFN- γ does not require de novo protein synthesis, in contrast to MHC class II genes, since tyrosine phosphorylation of STAT1 was shown to be the necessary and sufficient signal for IFN- γ triggering of Fc γ RI transcription in myeloid cells, through the GAS (γ -IFN-activated sequence) promoter element (33-35). Activation of STAT1 through both type I (IFN- α and IFN- β) and type II (IFN- γ) IFN signaling pathways allowed to explain their synergism in antiviral activity and transcriptional activation of the 2'-5'-A synthetase gene (36, 37) but did not provide clues for a possible antagonism at the transcriptional level. However, Lu et al. (38) recently demonstrated that IFN- β blocking of IFN- γ induction of MHC class II gene expression occurred downstream of class II *trans*-activator (CIITA) mRNA induction by IFN- γ , and hence downstream of JAK/STAT signalization, which explains the observed gene specificity of IFN- β antagonism.

In this report, we demonstrate the ability of IFN- β to down-regulate IFN- γ -induced Fc γ RI surface expression in peripheral blood monocytes, in a dose- and time-dependent manner. This decrease of surface expression did not correlate with a reduction of Fc γ RI mRNA levels, suggesting a posttranscriptional effect of IFN- β . In addition, we demonstrated that the observed down-regulation of Fc γ RI surface expression was physiologically relevant, since the strong increase in Fc γ receptor-triggered respiratory burst in IFN- γ -treated cells was almost completely abrogated when IFN- β was added simultaneously, indicating that down-regulation of Fc γ RI surface expression correlated with diminished cellular signaling through Fc γ RI. The same antagonism between both IFNs on Fc γ RI surface expression was observed in peripheral blood monocytes obtained from multiple sclerosis patients, and inhibition by IFN- β was even more pronounced, as compared with healthy controls.

Materials and Methods

Patients

Blood was obtained from 10 Caucasian patients (7 females) with clinically definite multiple sclerosis (39). The age range was 22 to 50 years. Seven patients were examined during exacerbation or the chronic progressive phase of MS and three patients during remission. All patients had developed moderate to severe disability. None had received immunosuppressive drugs in the previous 3 mo. The study protocol was approved by the ethics committee of the Pitié-Salpêtrière Hospital, and an informed consent was obtained from all patients.

Cell isolation and culture

PBMC were separated by density gradient centrifugation (Ficoll-Paque, Pharmacia, Uppsala, Sweden) of cytopheresis residues obtained from normal blood donors (age range 18-55 yr). Monocytes were isolated by 1-h adherence on plastic in culture medium (RPMI 1640 supplemented with 10% FCS, Life Technologies, Paisley, Scotland), followed by extensive washing with PBS, which yielded >90% CD14-positive cells, as determined by FACS analysis. For small-scale preparations (patients and controls), approximately 20 ml of blood was diluted with an equal volume of PBS and processed as above. Cell recoveries (as assessed by trypan blue staining) were always >90% of initial cell counts for untreated as well as IFN-treated monocytes cultured for up to 72 h.

Cytokines and cytokine assay

Recombinant human (rHu) IFN- β (sp. act. 4×10^8 U/mg protein) was a gift of Ares-Serono (Geneva, Switzerland); rHuIFN- γ (sp. act. 2×10^7 U/mg protein) was a gift from Roussel-Uclaf (Romainville, France). Biologic activity of IFNs was determined on WISH cells using vesicular stomatitis virus as a challenge. TNF was quantified in a cytotoxicity assay using L929 cells in the presence of actinomycin D. IL-6 was quantified by measuring its hybridoma growth factor activity upon 7TD.1 cells.

Antibodies

Anti-Fc γ RI (clone 32.2), anti-Fc γ RII (clone IV.3), and anti-Fc γ RIII (clone 3G8) mAbs, either purified or FITC-labeled were purchased from Medarex

(West Lebanon, NH). Anti-Fc γ RI (clone 22), control isotypes, anti-CD14 (clone RMO52), anti- α_4 integrin (clone HP2/1), anti-CD11b (clone BEAR1) mAbs, and goat anti-mouse FITC-conjugated F(ab') $_2$ were obtained from Immunotech (Marseille, France). Anti-CD18 (clone MHM23) mAb was obtained from Dako (Glostrup, Denmark). The polyclonal Ab directed against FcR γ -chain was a gift from Dr. J. P. Kinet (Beth Israel Hospital, Boston, MA).

Flow cytometry

Cells were immunostained as previously described (40), except that Triton X-100 was substituted for saponin to permeabilize cells. Analysis was performed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) using Lysis II software. Results are expressed as mean fluorescence intensity (MFI), which was corrected by subtraction of values of isotype-matched controls for each data point. The percentage of inhibition of IFN- γ -induced Fc γ RI expression by IFN- β was calculated as follows: % inhibition = $(1 - [(MFI \text{ IFN-}\beta + \text{IFN-}\gamma\text{-treated cells} - MFI \text{ control cells}) / (MFI \text{ IFN-}\gamma\text{-treated cells} - MFI \text{ control cells})]) \times 100$.

RNA analysis

Total RNA was extracted using the acid guanidinium phenol chloroform method (41). RNA (5-15 μ g) was run on a formaldehyde-containing 1% agarose gel, transferred onto a nylon membrane (Biodyne Poly-Labs, Paris, France), and hybridized with the appropriate probe (1.5×10^6 cpm/ml), which had been labeled using [α - 32 P]dCTP (Dupont de Nemours, NEN, Boston, MA) and a random primer labeling kit (Amersham-France, Les Ulis, France).

Hybridizations were conducted in 50% formamide, $5 \times$ standard saline phosphate/EDTA (SSPE), 0.5% SDS, $5 \times$ Denhardt's, and 200 μ g/ml denatured salmon sperm DNA at 42°C, followed by washing to a final stringency of $0.2 \times$ SSPE at temperatures between 55 and 65°C. Resulting bands were quantified using a PhosphorImager system (Molecular Dynamics, Sunnyvale, CA) and normalized against corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts.

Superoxide assay

Production of superoxide was measured spectrophotometrically by reduction of ferricytochrome *c* that was inhibited by superoxide dismutase, as previously described (42).

Statistical analysis

All results are expressed as mean \pm SEM. Statistical evaluation of data was performed using a two-tailed Student *t* test.

Results

IFN- β down-regulates IFN- γ -induced surface expression of Fc γ RI in peripheral blood monocytes of normal donors

In initial experiments, freshly isolated peripheral blood monocytes from healthy controls were cultured overnight (16-18 h) in the presence of IFN- β , IFN- γ , or a combination of both, and cell surface expression of Fc γ RI was quantified by flow cytometry, in parallel with common monocyte surface molecules. Typical FACS profiles are shown in Figure 1. IFN- γ strongly induced Fc γ RI surface expression, which was markedly inhibited by simultaneous addition of IFN- β . Interestingly, addition of IFN- β alone had either no or a slight enhancing effect on Fc γ RI expression. Similar results were obtained with both anti-Fc γ RI mAbs (clone 32.2 and 22), as well as with monomeric human IgG1 (data not shown), the latter demonstrating that IFN- β did not change Fc γ RI binding capacity for its ligand but merely reduced the number of surface receptors.

As shown in Figure 2A, down-regulation by IFN- β of IFN- γ -induced Fc γ RI was statistically highly significant ($67 \pm 4\%$ inhibition for 20 independent donors, $p = 0.0008$). No significant modulation of the low affinity Fc receptor (Fc γ RII, CD32) was observed following overnight stimulation with either IFN (Fig. 2B). Similarly, surface expression of CD11b, CD14, CD18, or α_4 integrin remained unchanged under our experimental conditions (data not shown), which confirmed that the observed effect of IFN- β and IFN- γ was specific for the Fc γ RI molecule and not due

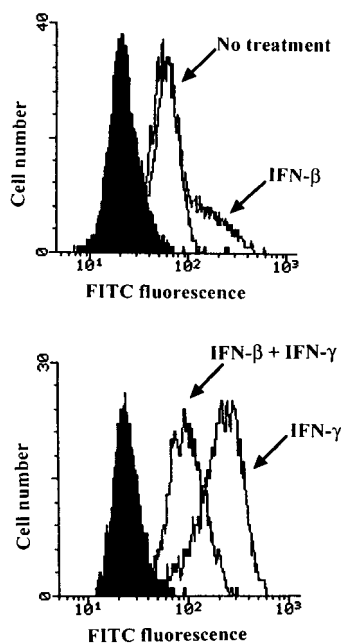


FIGURE 1. IFN- β down-regulates IFN- γ -induced surface expression of Fc γ RI in peripheral blood monocytes of normal donors. Cells were cultured for 16 h in the absence or presence of 500 U/ml of IFN- β , 50 U/ml of IFN- γ , or both, followed by immunostaining with FITC-labeled isotype control (filled histograms) or FITC-labeled anti-Fc γ RI-FITC mAb (clone 32.2, open histograms). Typical FACS profiles are shown; highly similar results were obtained from 20 independent donors.

to general changes in cell size or membrane properties induced by IFNs.

Down-regulation by IFN- β of IFN- γ -induced surface expression of Fc γ RI in human peripheral blood monocytes is dose- and time-dependent

The antagonistic effect of IFN- β on IFN- γ induction of Fc γ RI was dose dependent. Although absolute levels of Fc γ RI varied between different donors, strongest inhibition was generally observed at a 10:1 IFN- β /IFN- γ ratio in antiviral units (Fig. 3), which corresponds to a 1:2 molar ratio, however. Lower ratios were still ef-

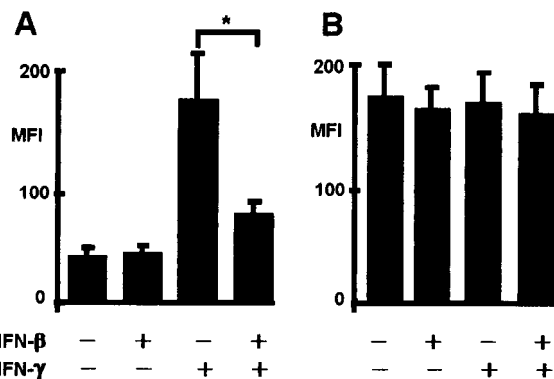


FIGURE 2. Down-regulation of IFN- γ -induced Fc γ RI surface expression by IFN- β is statistically significant and specific. Cells were cultured overnight (16-18 h) in the absence or presence of 500 U/ml of IFN- β , 50 U/ml of IFN- γ , or both, immunostained with (A) FITC-labeled anti-Fc γ RI (clone 32.2) or (B) anti-Fc γ RII mAbs, and analyzed by FACS. Data are represented as MFI \pm SEM of (A) 20 (* p = 0.0008) and (B) 5 independent experiments.

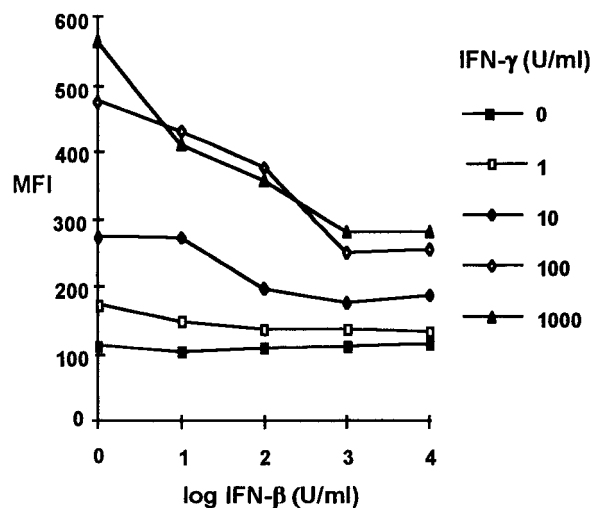


FIGURE 3. Down-regulation of IFN- γ -induced Fc γ RI surface expression by IFN- β is dose dependent. Cells were cultured for 16 h in the absence or presence of increasing amounts of IFN- β , IFN- γ , or both, immunostained with FITC-labeled anti-Fc γ RI mAb (clone 32.2), and analyzed by FACS. Data are representative of five experiments.

fective; e.g., 10 U/ml of IFN- β caused a 28% decrease in Fc γ RI surface level induced by 1,000 U/ml of IFN- γ (Fig. 3). Fc γ RI induction by IFN- γ was nearly maximum at 100 U/ml, while inhibition by IFN- β reached a plateau at 1,000 U/ml. For highest reproducibility between donors, half-maximal doses of IFN- γ and IFN- β (50 U/ml and 500 U/ml, respectively) were used in all additional experiments.

As shown in Figure 4, Fc γ RI down-regulation by IFN- β was also time dependent. At each time point, IFN- β was capable of significantly down-regulating IFN- γ induction of Fc γ RI surface expression, but there was a gradual increase of approximately 50% at 12 h to >90% inhibition at 72 h of culture (Fig. 4, *inner graph*).

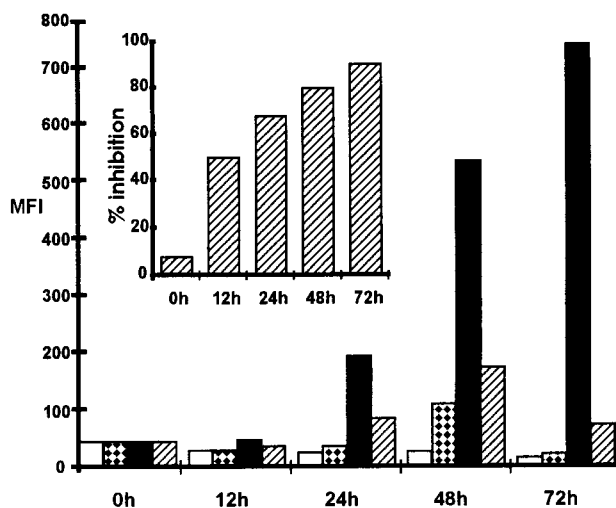


FIGURE 4. Down-regulation of IFN- γ -induced Fc γ RI surface expression by IFN- β is time dependent. Cells were cultured in the absence (open bars) or presence of 500 U/ml IFN- β (stippled bars), 50 U/ml IFN- γ (filled bars), or both (hatched bars) for different time periods, immunostained with FITC-labeled anti-Fc γ RI mAb (clone 32.2), and analyzed by FACS. The *inset* graph shows percent inhibition of Fc γ RI expression in cells treated with IFN- β and IFN- γ , as compared with cells treated with IFN- γ alone. Data are representative of three experiments.

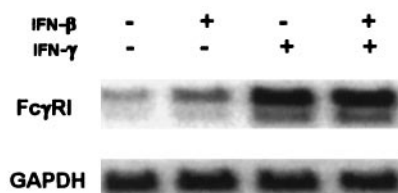


FIGURE 5. IFN- β does not down-regulate IFN- γ -induced Fc γ RI mRNA in human peripheral blood monocytes. Cells were cultured for 16 h in the presence of 500 U/ml IFN- β , 50 U/ml IFN- γ , or both. RNA was extracted and subjected to Northern blot analysis using Fc γ RI cDNA as a probe. Bands were quantified using PhosphorImager software and normalized for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. Data are representative of eight experiments.

These kinetics reveal a rather delayed effect of IFN- β , since maximal inhibition of IFN- γ action was observed only after 3 days of incubation.

Therefore, we considered the possibility that preincubation with IFN- β might enhance or accelerate its antagonizing effect on IFN- γ . Monocytes preincubated with IFN- β for 2 h before addition of IFN- γ showed a slightly stronger decrease in Fc γ RI expression ($80 \pm 17\%$, $n = 3$) as compared with cells to which both IFNs were simultaneously added ($67 \pm 4\%$, Fig. 2A); this difference was not significant, however. Moreover, addition of IFN- β 2 h after IFN- γ was still effective in down-regulating Fc γ RI expression, albeit to a lesser extent ($41 \pm 19\%$, $n = 3$).

IFN- β does not down-regulate IFN- γ induction of Fc γ RI mRNA in human peripheral blood monocytes

We next examined whether down-regulation of Fc γ RI at the cell surface would be reflected by decreased Fc γ RI mRNA levels, since Fc γ RI gene induction by IFN- γ had previously been shown to occur at the transcriptional level (33-35). Fc γ RI mRNA was quantified by Northern blot analysis, using a cDNA probe that hybridizes to all transcripts of the three Fc γ RIA, -IB and -IC genes, but the Fc γ RIA mRNA, which encodes the bona fide high affinity receptor, is strongly predominating. Surprisingly, there was no difference in Fc γ RI mRNA between monocytes treated with IFN- γ or with IFN- β + IFN- γ for 16 h (Fig. 5), the time point at which surface expression was significantly down-regulated. When looking at earlier mRNA kinetics (2 to 8 h), again no correlation was found between Fc γ RI mRNA and surface expression (measured in parallel experiments at 16 h from the same donors) in IFN- β + IFN- γ -treated cells, in contrast to IFN- γ -treated cells (data not shown), suggesting a posttranscriptional effect of IFN- β .

IFN- β down-regulates total cellular IFN- γ -induced Fc γ RI protein, but not FcR-associated γ -chain in human peripheral blood monocytes

A possible mechanism for IFN- β to decrease Fc γ RI surface expression without altering steady state mRNA levels might be intracellular sequestration, which has been demonstrated for IFN- β -induced down-regulation of transferrin receptor surface expression in human macrophages (43). Therefore, untreated and IFN-treated monocytes were fixed and permeabilized to allow intracellular staining to quantify total cellular Fc γ RI protein content by FACS. As shown in Table I, IFN- β decreased total IFN- γ -induced Fc γ RI protein levels, in permeabilized as well as unpermeabilized cells, contesting intracellular sequestration of Fc γ RI.

Another conceivable mechanism through which IFN- β might block Fc γ RI expression might be through decreasing FcR γ -chain levels, since γ -chain expression has recently been demonstrated to

Table I. IFN- β down-regulates IFN- γ -induced surface as well as intracellular Fc γ RI expression^a

Cell Treatment	Intact Cells	Permeabilized Cells
Untreated	39 \pm 3	59 \pm 6
IFN- β	36 \pm 9	58 \pm 11
IFN- γ	111 \pm 15	88 \pm 7
IFN- β + IFN- γ	51 \pm 2	53 \pm 15

^a Cells were cultured for 16 h in the absence or presence of 500 U/ml of IFN- β , 50 U/ml of IFN- γ , or both, and either left untreated (intact cells) or permeabilized as described in *Materials and Methods*, before immunostaining with FITC-labeled anti-Fc γ RI (clone 32.2) mAb and analysis by FACS. Data are represented as MFI \pm SEM of three different donors.

be essential for both Fc γ RI surface expression and function (44). However, γ -chain mRNA and protein levels did not alter upon treatment with IFN- β , IFN- γ , or both together (results not shown), arguing against a γ -chain-mediated effect of IFN- β .

IFN- β down-regulates IFN- γ -induced Fc γ receptor-mediated respiratory burst and proinflammatory cytokine secretion in human peripheral blood monocytes

To substantiate if the observed differences in Fc γ RI surface levels corresponded to a different functional status of IFN-treated cells, Fc γ receptor-triggered respiratory burst was measured by determining superoxide production in a sensitive ferricytochrome *c* reduction assay. As shown in Figure 6A, binding and subsequent cross-linking of murine IgG2a, which binds with high affinity to Fc γ RI, as well as anti-Fc γ RI mAb 32.2, resulted in a three- to fourfold increase in superoxide production in IFN- γ -treated cells. This increase was almost completely abrogated in IFN- β + IFN- γ -treated cells (Fig. 6A), in agreement with the observed decrease in surface expression. Likewise, IFN- β efficiently antagonized the IFN- γ -induced increase in secretion of TNF and IL-6, triggered by Fc γ RI cross-linking, followed by overnight culture (Fig. 6B).

IFN- β down-regulates IFN- γ -induced surface expression of Fc γ RI in peripheral blood monocytes of MS patients

By reason of its clinical significance in MS, we wondered if in vitro supplementation of IFN- β would have the same inhibitory potential on IFN- γ induction of Fc γ RI in monocytes from MS patients. As shown in Figure 7A, typical FACS profiles of monocytes from a representative MS patient are highly similar to those obtained from healthy controls (Fig. 1), except for the lower cell number in patient samples, due to the limited quantity of blood available. IFN- β was found to significantly down-regulate IFN- γ induction of Fc γ RI in monocytes of all 10 MS patients tested ($82 \pm 11\%$ inhibition, $p = 0.01$), as shown in Figure 7B. Inhibition by IFN- β was even greater in monocytes of MS patients ($82 \pm 11\%$, range 38-163%), as compared with healthy controls ($67 \pm 4\%$, range 35-95%). Although the SEM was higher in patients than in controls, this difference approached statistical significance ($p = 0.07$).

Discussion

The clinically opposed effects of IFN- β and IFN- γ in MS have been clearly demonstrated (1-4), but data on their antagonistic action at the molecular level have so far been limited to MHC class II expression (5-7). The aim of this study was to examine the possible effect of IFN- β on the high affinity Fc γ receptor, which is not only a key effector molecule in monocyte/macrophage metabolism but also the prototype of an immediate early IFN- γ -induced gene, by virtue of its extensively studied GAS promoter element.

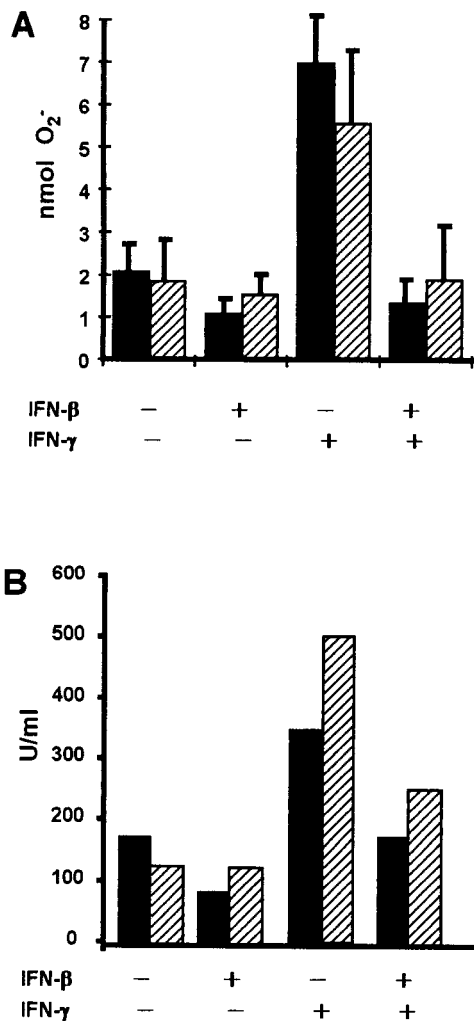


FIGURE 6. IFN- β down-regulates IFN- γ -induced Fc γ RI receptor-triggered respiratory burst and proinflammatory cytokine secretion in human peripheral blood monocytes. **A**, Cells were incubated in the absence or presence of 500 U/ml IFN- β , 50 U/ml IFN- γ , or both for 24 h, washed twice with PBS, incubated for 1 h at 37°C with 10 μ g/ml mouse IgG2a (black bars) or 5 μ g/ml anti-Fc γ RI mAb 32.2 (hatched bars), washed, and resuspended in superoxide assay buffer (42) in the presence of goat anti-mouse F(ab')₂ fragments and incubated for 1 h at 37°C. Superoxide production was quantified spectrophotometrically, as previously described (42). Data represent the mean \pm SEM of three experiments. **B**, Cells were incubated in the absence or presence of 500 U/ml IFN- β , 50 U/ml IFN- γ , or both for 24 h, washed twice with PBS, incubated for 1 h at 37°C with 5 μ g/ml anti-Fc γ RI mAb 32.2, washed, and stimulated overnight in the presence of goat anti-mouse F(ab')₂ fragments. TNF (black bars) and IL-6 (hatched bars) were quantified in cell-free supernatants by sensitive bioassays. Data are representative of three experiments.

We were able to demonstrate significant, dose-dependent down-regulation of IFN- γ -induced Fc γ RI surface expression by IFN- β . This down-regulation by IFN- β was also time dependent, but with apparently slow kinetics: 50% inhibition was observed at 12 h already, but maximal inhibition (>90%) required 72 h (Fig. 4). Preincubation with IFN- β 2 h before IFN- γ moderately enhanced its inhibitory effect at 16 h, but IFN- β added 2 h after IFN- γ was still effective, albeit to a lesser extent, at 16 h. At 72 h of treatment, however, the percentage of inhibition was similar (>90%) for cells to which IFN- β was added 2 h before, simultaneously, or even 2 h after IFN- γ . This time-dependent increase of IFN- β inhibition and the very limited effect of short-term sequential addition were

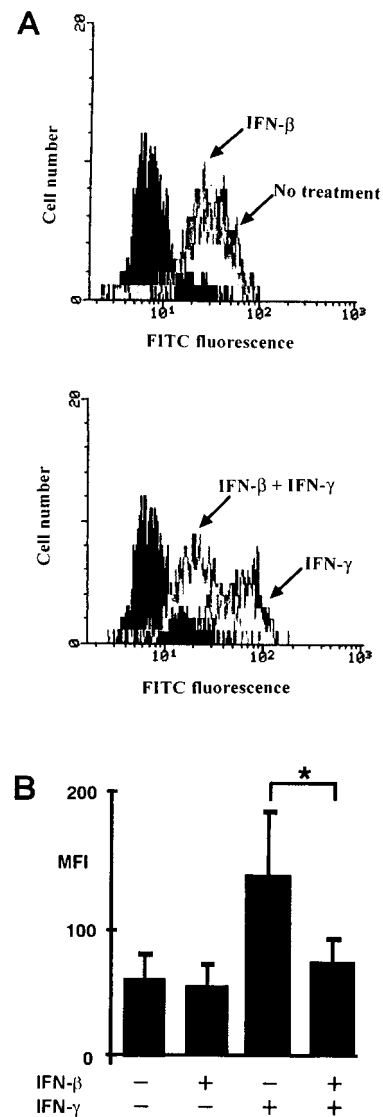


FIGURE 7. IFN- β down-regulates IFN- γ -induced surface expression of Fc γ RI in peripheral blood monocytes of multiple sclerosis patients. Cells were cultured for 16 h in the absence or presence of 500 U/ml of IFN- β , 50 U/ml of IFN- γ , or both, followed by immunostaining with FITC-labeled isotype control (filled histograms in **A**) or FITC-labeled anti-Fc γ RI-FITC mAb (open histograms in **A**). FACS profiles of monocytes from a representative patient are shown in **A**; similar profiles were obtained from all MS patients tested ($n = 10$). The MFI \pm SEM of 10 patients is given in **B**; * $p = 0.01$.

somehow unexpected, considering that the necessary and sufficient signal for Fc γ RI gene induction by IFN- γ , i.e., tyrosine phosphorylation of STAT1, occurs within minutes following receptor binding (33-35). Moreover, IFN- β did not decrease IFN- γ -induced Fc γ RI mRNA levels (Fig. 5). Taken together, these data strongly suggest that IFN- β does not exert its antagonistic effect on IFN- γ induction of Fc γ RI by blocking JAK/STAT signaling and early transcription, but through a posttranscriptional mechanism, most probably Fc γ RI specific. We cannot exclude the possibility that IFN- β might down-regulate IFN- γ -induced transcription and simultaneously increase Fc γ RI mRNA stability, resulting in equal mRNA levels. This assumption is highly unlikely, however, since IFN- β by itself activates STAT1 (45) and slightly, but significantly, induces Fc γ RI mRNA (Fig. 5). Furthermore, the absence of correlation between high Fc γ RI mRNA levels and low surface

expression in cells treated with IFN- β + IFN- γ clearly demonstrates that the physiologically important down-regulation occurs downstream of mRNA accumulation. To our knowledge, this is the first demonstration of IFN- β and IFN- γ antagonistically regulating the expression of the same protein at two different molecular levels.

A possible posttranslational mechanism through which IFN- β might down-regulate Fc γ RI surface expression is through intracellular sequestration, since IFN- β has been previously shown to down-regulate transferrin receptor surface expression in human macrophages by keeping these receptors in intracellular compartments (43). However, as shown in Table I, IFN- β decreased total IFN- γ -induced Fc γ RI protein levels, in intact as well as in permeabilized cells, i.e., intracellular as well as at the cell surface. This finding was confirmed by confocal microscopy (not shown), which allowed us to exclude the possibility of IFN- β -induced intracellular sequestration of Fc γ RI.

Since FcR γ -chain expression has been recently revealed to be essential for both Fc γ RI surface expression and function (44), another conceivable mechanism of IFN- β action might be an indirect one, i.e., blocking Fc γ RI expression by reducing the available γ -chain pool. However, γ -chain mRNA and protein levels did not alter upon treatment with IFN- β , IFN- γ , or both together (results not shown), arguing against a γ -chain-mediated effect of IFN- β . The observed decrease in Fc γ RI production might thus be due to an IFN- β -induced decrease in protein synthesis, i.e., a block in Fc γ RI mRNA translation, or to an IFN- β -induced protein degradation. To test the latter hypothesis, we have repeated our FACS analysis with untreated and IFN-treated monocytes cultured in the presence of a mixture of protease inhibitors. Addition of this mixture had no effect on either IFN- γ up-regulation or IFN- β down-regulation, arguing against IFN- β -enhanced Fc γ RI proteolysis. The strongest argument against IFN- β -induced degradation comes from delayed addition experiments, where IFN- β had to be added until up to 12 h after IFN- γ (i.e., the time needed for Fc γ RI to be expressed at the cell surface) to be fully effective. When monocytes were pretreated with IFN- γ for 24 or 48 h, IFN- β provoked only a minor decrease in Fc γ RI surface expression, which in fact corresponded to inhibiting a further increase by IFN- γ . In other words, IFN- β was ineffective in down-regulating IFN- γ -induced Fc γ RI already expressed at the cell surface, which ought to be the case if IFN- β were to induce Fc γ RI proteolysis. Accordingly, we favor the hypothesis of translational inhibition by IFN- β , which is currently under investigation.

This posttranscriptional regulation by IFN- β contrasts with the mechanism of action on Fc γ RI expression of other cytokines, such as IL-4 and IL-10, which both act at the transcriptional level. Down-regulation by IL-4 is mediated by activation of STAT6, which competes with STAT1 α for GAS binding (46), whereas up-regulation by IL-10 corresponds to STAT1 activation (47), as for IFN- γ . However, our results are concordant with those of Lu et al. (38), who demonstrated that blocking by IFN- β of IFN- γ -induced MHC class II gene expression occurs downstream of CIITA mRNA induction and hence downstream of JAK/STAT activation by IFN- γ . This implies a high selectivity of IFN- β -IFN- γ antagonism, apparently limited to MHC class II and Fc γ RI genes, in contrast to IL-4, which abrogates IFN- γ signaling through STAT1 α and hence causes a generalized block of GAS-mediated gene induction and Fc γ RI, as well as IFN regulatory factor (IRF)-1 guanylate-binding protein (GBP), CIITA, tryptophanyl tRNA synthetase, and other IFN- γ -inducible genes. This gene-specificity of IFN- β action might be physiologically interesting in fine-tuning an IFN- γ -mediated inflammatory response by selectively blocking key activator molecules without hampering other essential func-

tions of IFN- γ , such as antimicrobial or antiviral activity, the latter being synergistically enhanced by IFN- β (36).

To substantiate if the observed differences in Fc γ RI expression were physiologically relevant, Fc γ RI-mediated respiratory burst and secretion of proinflammatory cytokines (TNF and IL-6) were quantified in untreated and IFN-treated cells. IFN- γ and IFN- β were indeed found to up- and down-regulate Fc γ RI-triggered respiratory burst, respectively, (Fig. 6A), as well as Fc γ RI-triggered secretion of TNF and IL-6 (Fig. 6B), demonstrating that Fc γ RI surface expression and function were tightly linked. Besides triggering of the respiratory burst and secretion of proinflammatory cytokines, cellular signaling through Fc γ RI comprises phagocytosis and Ab-dependent cytotoxicity (26). In addition, Fc γ RI is endowed with Ag presentation capacity, as demonstrated both in vitro (48) and in vivo (49). Accordingly, the high affinity Fc γ receptor can be incriminated in the pathogenesis of demyelination for its potency to mediate cellular as well as humoral autoimmune reactivity.

Finally, Fc γ RI is predominantly expressed in cells of the monocyte-macrophage lineage, which are prevailing in MS lesions at later stages of the disease. Nevertheless, it remained to be demonstrated if the observed antagonistic effect of both IFNs could be reproduced in monocytes from MS patients, since MS monocytes have been shown to differ phenotypically and functionally from normal monocytes (Ref. 50, and references therein). We found that in vitro supplementation of IFN- β indeed inhibited IFN- γ -induced Fc γ RI surface expression in monocytes of all ten patients tested and that this inhibition was even more pronounced ($82 \pm 11\%$) than in healthy controls ($67 \pm 4\%$) when monocytes were cultured for 24 h.

In conclusion, we provide strong evidence that Fc γ RI might be considered, in addition to MHC class II, as a physiologically relevant target protein for antagonistic regulation by IFN- γ and IFN- β , which may partially explain the beneficial effect of IFN- β in multiple sclerosis.

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References

- Panitch, H. S., R. L. Hirsch, A. S. Haley, and K. P. Johnson. 1987. Exacerbations of multiple sclerosis in patients treated with γ -IFN. *Lancet* *i*:893.
- IFN- β Multiple Sclerosis Study Group. 1993. Interferon- β 1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* *43*:656.
- Paty, D., D. Li, UBC MS/MRI Study Group, and IFN- β Multiple Sclerosis Study Group. 1993. Interferon- β 1b is effective in relapsing-remitting multiple sclerosis. II. MRI results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* *43*:662.
- Jacobs, L. D., D. L. Cookfair, R. A. Rudick, R. M. Herndon, J. R. Richert, A. M. Salazar, J. S. Fischer, D. E. Goodkin, C. V. Granger, J. H. Simon, J. J. Alam, D. M. Bartsch, D. N. Bourdette, J. Braiman, C. M. Braunschweig, M. E. Coats, S. L. Cohan, D. S. Dougherty, R. P. Kinkel, M. K. Mass, F. E. Munschauer, R. L. Priore, P. M. Pulicino, B. J. Scherokman, B. Weinstock-Guttman, R. H. Whitham, and the Multiple Sclerosis Collaborative Research Group. 1996. Intramuscular IFN- β 1a for disease progression in relapsing multiple sclerosis. *Ann. Neurol.* *39*:285.
- Ling, P. D., M. K. Warren, and S. N. Vogel. 1985. Antagonistic effect of IFN- β on the IFN- γ -induced expression of Ia antigen in murine macrophages. *J. Immunol.* *135*:1857.

6. Ibane, K., M. Kitaura, T. Kato, Y. Watanabe, Y. Kawade, and S. Maramatsu. 1986. Contrasting effects of $\alpha\beta$ - and γ -IFNs on expression of macrophage Ia antigens. *J. Exp. Med.* 163:1030.
7. Barna, B. P., S. M. Chou, B. Jacobs, B. Yen-Lieberman, and R. M. Ransohoff. 1989. Interferon- β impairs induction of HLA-DR antigen expression in cultured adult human astrocytes. *J. Neuroimmunol.* 23:45.
8. Weinstock-Guttman, B., R. Ransohoff, R. Kinkel, and R. Rudick. 1995. The IFNs: biological effects, mechanisms of action, and use in multiple sclerosis. *Ann. Neurol.* 37:7.
9. Fabry, Z., C. S. Raine, and M. N. Hart. 1994. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. *Immunol. Today* 15:219.
10. Zhang, J., L. Howard, H. L. Weiner, and D. A. Hafler. 1992. Autoreactive T cells in multiple sclerosis. *Int. Rev. Immunol.* 9:183.
11. Hohfeld, R., M. Londei, L. Massacesi, and M. Salvetti. 1995. T-cell autoimmunity in multiple sclerosis. *Immunol. Today* 16:259.
12. Steinman, L. 1996. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 85:299.
13. Pettinelli, C. B., and D. E. McFarlin. 1981. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement for Lyt-1⁺2⁻ T lymphocytes. *J. Immunol.* 127:1420.
14. Zamvil, S. S., P. A. Nelson, D. J. Mitchell, R. L. Knobler, R. B. Fritz, and L. Steinman. 1985. Encephalitogenic T cell clones specific for myelin basic protein: an unusual bias in antigen recognition. *J. Exp. Med.* 162:21.
15. Genain, C. P., D. Lee Parritz, M. N. Nguyen, L. Massacesi, N. Joshi, R. Ferrante, K. Hoffman, M. Moseley, N. L. Letvin, and S. L. Hauser. 1994. In healthy primates, circulating autoreactive T cells mediate autoimmune disease. *J. Clin. Invest.* 94:1339.
16. Willenberg, D. O., and S. J. Prowse. 1983. Immunoglobulin-deficient rats fail to develop experimental allergic encephalomyelitis. *J. Neuroimmunol.* 5:99.
17. Genain, C. P., M. H. Nguyen, N. L. Letvin, R. Pearl, R. L. Davis, M. Adelman, M. B. Lees, C. Linington, and S. L. Hauser. 1995. Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. *J. Clin. Invest.* 96:2966.
18. Panitch, H. S., C. J. Cooper, and K. P. Johnson. 1980. CSF antibody to myelin basic protein: measurement in patients with multiple sclerosis and subacute sclerosing panencephalitis. *Arch. Neurol.* 37:206.
19. Warren, K. G., I. Gatz, E. Johnson, and B. Mielke. 1994. Anti-myelin basic protein and anti-proteolipid protein specific forms in multiple sclerosis. *Ann. Neurol.* 35:280.
20. Merrill, J. E., D. A. Kono, J. Clayton, D. G. Ando, D. R. Hinton, and F. M. Hofman. 1992. Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. *Proc. Natl. Acad. Sci. USA* 89:574.
21. Cuzner, M. L., G. M. Hayes, J. Newcombe, and M. N. Woodroffe. 1988. The nature of inflammatory components during demyelination in multiple sclerosis. *J. Neuroimmunol.* 20:203.
22. Goldenberg, P. Z., E. E. Kwon, J. A. Benjamins, J. N. Whitaker, R. H. Quarles, and J. W. Prineas. 1989. Opsonization of normal myelin by anti-myelin antibodies and normal serum. *J. Neuroimmunol.* 23:157.
23. Mosley, K., and M. L. Cuzner. 1996. Receptor-mediated phagocytosis of myelin by macrophages and microglia: effect of opsonization and receptor blocking agents. *Neurochem. Res.* 21:481.
24. Ulvestad, E., K. Williams, C. Vedeler, J. Antel, H. Nyland, S. Mork, and R. Matre. 1994. Reactive microglia in multiple sclerosis lesions have an increased expression of receptors for the Fc part of IgG. *J. Neurol. Sci.* 121:125.
25. Scolding, N. J., and D. A. S. Compston. 1991. Oligodendrocyte-macrophage interactions in vitro triggered by specific antibodies. *Immunology* 72:127.
26. van de Winkel, J. G. J., and P. J. A. Capel. 1993. Human IgG Fc receptors heterogeneity: molecular aspects and clinical implications. *Immunol. Today* 14:215.
27. Ruuls, S. R., J. Bauer, K. Sontrop, I. Huitinga, B. A. 't Hart, and C. D. Dijkstra. 1995. Reactive oxygen species are involved in the pathogenesis of experimental allergic encephalomyelitis in Lewis rats. *J. Neuroimmunol.* 56:207.
28. Raine, C. S. 1994. The Dale E. McFarlin memorial lecture: the immunology of the multiple sclerosis lesion. *Ann. Neurol.* 36:S61.
29. Selmaj, K., C. S. Raine, B. Cannella, and C. F. Brosnan. 1991. Identification of lymphotoxin and TNF in multiple sclerosis lesions. *J. Clin. Invest.* 87:949.
30. Beck, J., P. Rondot, L. Catinot, E. Falcoff, H. Kirchner, and J. Wietzerbin. 1988. Increased production of IFN- γ and TNF precedes clinical manifestations in multiple sclerosis: do cytokines trigger off exacerbations? *Acta Neurol. Scand.* 78:318.
31. Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark. 1994. JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264:1415.
32. Ihle, J. N. 1996. STATs: signal transducers and activators of transcription. *Cell* 84:331.
33. Pearce, R. N., R. Feinman, K. Shuai, J. E. Darnell, Jr., and J. V. Ravetch. 1993. Interferon- γ -induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of transcription factor ISGF3. *Proc. Natl. Acad. Sci. USA* 90:4314.
34. Perez, C., J. Wietzerbin, and P. Benech. 1993. Two cis-DNA elements involved in myeloid-cell-specific expression and gamma IFN (IFN- γ) activation of the human high-affinity Fc γ receptor gene: a novel IFN regulatory mechanism. *Mol. Cell. Biol.* 13:2182.
35. Igarashi, K. I., M. David, A. Larner, and D. S. Finbloom. 1993. In vitro activation of a transcription factor by gamma IFN requires a membrane-associated tyrosine kinase and is mimicked by vanadate. *Mol. Cell. Biol.* 13:3984.
36. Olesjak, E., and W. E. Stewart. 1985. Potentiation of the antiviral and anticellular activities of IFNs by mixtures of HuIFN- γ and HuIFN- α or HuIFN- β . *J. Interferon Res.* 5:361.
37. Vaiman, D., S. Pietrovskoy, B. Cohen, P. Benech, and J. Chebath. 1990. Synergism of type I and type II IFNs in stimulating the activity of the same DNA enhancer. *FEBS Lett.* 265:12.
38. Lu, H., J. L. Riley, G. T. Babcock, M. Huston, G. R. Stark, J. M. Boss, and R. M. Ransohoff. 1995. Interferon (IFN) β acts downstream of IFN- γ -induced class II trans-activator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kDa DNA-binding protein, ISGF3- γ . *J. Exp. Med.* 182:1517.
39. Poser, C. M., D. W. Paty, L. Scheinberg, W. I. McDonald, F. A. Davis, G. C. Ebers, K. P. Johnson, W. A. Sibley, D. H. Silberberg, and W. W. Tourtelotte. 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann. Neurol.* 13:227.
40. Bauvois, B., J. Van Weyenbergh, D. Rouillard, and J. Wietzerbin. 1996. TGF- β -stimulated adhesion of human mononuclear phagocytes to fibronectin and laminin is abolished by IFN- γ : dependence on $\alpha_5\beta_1$ and β_2 integrins. *Exp. Cell Res.* 222:209.
41. Chomczynski, P., and N. Sacchi. 1987. Single-step method for RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.
42. Lipinski, P., and H. Retmanska. 1996. Horse spleen ferritin inhibits superoxide production by equine blood monocytes in vitro. *Free Radic. Biol. Med.* 20:729.
43. Testa, U., L. Conti, N. M. Sposi, B. Varano, E. Tritarelli, W. Malorni, P. Samoggia, G. Rainaldi, C. Peschle, F. Belardelli, and S. Gessani. 1995. IFN- β selectively down-regulates transferrin receptor expression in human peripheral macrophages by a post-translational mechanism. *J. Immunol.* 155:427.
44. van Vugt, M. J., I. A. F. M. Heijnen, P. J. A. Capel, S. Y. Park, C. Ra, T. Saito, J. S. Verbeek, and J. G. J. van de Winkel. 1996. FcR γ -chain is essential for both surface expression and function of human Fc γ RI (CD64) in vivo. *Blood* 87:3593.
45. Petricoin, E., III, M. David, K. Igarashi, C. Benjamin, L. Ling, S. Goelz, D. S. Finbloom, and A. C. Larner. 1996. Inhibition of alpha IFN but not gamma IFN signal transduction by phorbol esters is mediated by a tyrosine phosphatase. *Mol. Cell. Biol.* 16:1419.
46. Kotanides, H., and N. C. Reich. 1993. Requirement of tyrosine phosphorylation for rapid activation of a DNA binding factor by IL-4. *Science* 262:1265.
47. Larner, A. C., M. David, G. M. Feldman, K. I. Igarashi, R. H. Hackett, D. S. A. Webb, S. M. Sweitzer, E. F. Petricoin, III, and D. S. Finbloom. 1993. Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. *Science* 262:1730.
48. Gosselin, E. J., K. Wardwell, D. R. Gosselin, N. Alter, J. L. Fisher, and P. M. Guyre. 1992. Enhanced antigen presentation using human Fc γ receptor (monocyte/macrophage)-specific immunogens. *J. Immunol.* 149:3477.
49. Heijnen, I. A. F. M., M. J. Van Vugt, N. A. Fanger, R. F. Graziano, T. P. M. de Wit, F. M. A. Hofhuis, P. M. Guyre, P. J. A. Capel, J. S. Verbeek, and J. G. J. van de Winkel. 1996. Antigen targeting to myeloid-specific human Fc γ RI/CD64 triggers enhanced antibody responses in transgenic mice. *J. Clin. Invest.* 97:331.
50. Dore-Duffy, P., C. Donovan and R. F. Todd, III. 1992. Expression of monocyte activation antigen Mo3 on the surface of peripheral blood monocytes from patients with multiple sclerosis. *Neurology* 42:1609.