IFN-β and TGF-β differentially regulate IL-12 activity in human peripheral blood mononuclear cells

Johan Van Weyenbergh a,* Maria da Purificação P. Silva a, André Báfrica a, Silvia Cardoso a, Juana Wietzerbin b, Manoel Barral-Netto a

a Laboratório de Imun-regulação e Microbiologia, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão 121, 40295-001 Salvador, Bahia, Brazil
b U365 INSERM, Institut Curie, Section de Recherche, Paris, France

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Abstract

Both IFN-β and TGF-β have demonstrated their ability to antagonize several of the stimulatory activities of IFN-γ on human macrophages, thereby classifying them as Th2-like. Aiming at a further characterization of their role in Th1:Th2 development, we studied their possible interaction with IL-12, the key Th1 cytokine. We found that IFN-β by itself induced modest amounts of IFN-γ, but was able to synergize with IL-12 for IFN-γ induction. TGF-β, on the other hand, had no effect by itself and inhibited significantly the IL-12-induced IFN-γ secretion. The differential effect of IFN-β and TGF-β on IL-12 bioactivity was most pronounced upon IFN-γ synthesis, since IFN-β induced only marginal amounts of IL-10 and IL-12 and TGF-β diminished constitutive IL-10 production, while neither had a significant effect on TNF-α production. Although monocytes did not produce detectable IFN-γ with any of the stimuli, adherent cells were found to cooperate with non-adherent lymphocytes for maximal IFN-γ production. However, IL-18, a monocyte-derived IFN-γ-inducing cytokine able to synergize with IL-12, was undetectable in IFN-β or IFN-β + IL-12-stimulated cells. In conclusion, the ability of IFN-β to synergize with IL-12 for IFN-γ synthesis, without significant concomitant IL-10 production, suggest a strong boost to Th1 development, which seems to be IL-18-independent. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Antagonistic action of IFN-β and TGF-β towards IFN-γ has been demonstrated at the molecular level for several genes in human and murine monocytes/macrophages, including MHC class II [1], ICSBP [2], iNOS [3], z5 integrin [4] and FcγRI [5], which might allow their classification as Th2-like or ‘macrophage-deactivating’ cytokines. However, conflicting evidence has emerged in recent literature as for the role of IFN-β and TGF-β in IFN-γ production and Th1/Th2 development. First, IFN-α has been shown to increase the frequency of IFN-γ-producing human CD 4 + T cells [6], but does not sustain Th1 development [7]. Second,

IFN-β has been shown to interfere with IL-4 + GM-CSF-induced dendritic cell differentiation (DC1 phenotype), by down-regulating both their capacity to produce IL-12 [8,9] and their subsequent induction of IFN-γ production in co-cultured T-lymphocytes [8]. Accordingly, precursor cells of the DC2 phenotype (pDC2) have been shown to be the principal type I IFN-producing cells in human blood [10]. We have earlier shown that TGF-β [11] and IFN-β (Van Weyenbergh et al., manuscript in preparation) are able to increase parasite burden in Leishmania-infected human macrophages and to antagonize the protective effect of IFN-γ. Aiming at a further characterization of the role of both cytokines in Th1/Th2 development, we studied their possible interaction with IL-12, the key Th1 cytokine and upstream inducer of IFN-γ. We found that IFN-β by itself induced modest amounts of IFN-γ, but was able to synergize with IL-12 for IFN-γ induction.

* Corresponding author. Tel.: +55-71-3564320; fax: +55-71-3562155.
E-mail address: ejomaflo@svn.com.br (J. Van Weyenbergh).
TGF-β, on the other hand, had no effect by itself and inhibited significantly the IL-12-induced IFN-γ secretion. IFN-β and IL-12 have been described to induce IL-10 and TNF-α synthesis, a Th2- and a Th1-like cytokine, respectively. In our hands, however, IFN-β induced only marginal amounts of IL-10 and IL-12 diminished the constitutive IL-10 production, while neither had a significant effect on TNF-α production. At the cellular level, monocytes did not produce detectable IFN-γ with any of the stimuli, but adherent cells were found to cooperate with non-adherent lymphocytes for maximal IFN-γ production. This cooperation between the adherent and non-adherent cell populations was apparently not mediated by IL-18, a monocyte-derived IFN-γ-inducing cytokine known to synergize with IL-12 [12], since it was undetectable in IFN-β or IFN-β + IL-12-stimulated cells supernatants.

2. Materials and methods

2.1. Cell isolation and culture

Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation (Ficoll-Paque, Pharmacia, Uppsala, Sweden) of buffy coats obtained from normal blood donors. When required, monocytes and lymphocytes were purified by 30-min adherence on plastic in culture medium (RPMI 1640 without serum, GIBCO-BRL, Paisley, Scotland), followed by gentle washing with PBS and cultured in complete culture medium (supplemented with 10% fetal calf serum, GIBCO-BRL). Cell recoveries (as assessed by trypan blue staining) were always >90% of initial cell counts for untreated as well as cytokine-treated cells. Cell counts for untreated as well as cytokine-treated cells were always determined by trypan blue staining. IFN-γ was inhibited significantly the IL-12-induced IFN-γ.

2.2. Cytokines and cytokine assays

rHuIFN-β (specific activity, 4 × 10^8 U/mg protein) was a gift of Ares-Serono (Geneva, Switzerland). IL-12 was a gift of Wyeth-Ayerst (Paris, France). TGF-β1 was purchased from Genzyme (Cambridge, MA, USA). Interferon bioactivity was confirmed on WISH cells using vesicular stomatitis virus as a challenge. IFN-γ, TNF-α, IL-10 and IL-18 in culture supernatants were quantified using commercial ELISA kits (DuoSet, R&D, Pharmingen for IL-10, and MBL for IL-18).

2.3. Antibodies

Anti-CD 14, anti-CD 3, anti-CD 19, anti-CD 56 mAbs and control mouse isotypes were obtained from Immunotech (Marseille, France). Anti-IFN-γ mAb and rat control isotype were purchased from Pharmingen.

2.4. Flow cytometry

Cells were immunostained as described previously [4], except that Triton X-100 was substituted for saponin to permeabilize cells. Analysis on 10 000 cells per data point was performed on a FACSort flow cytometer (Becton Dickinson & Co., Mountain View, CA, USA) using CELLQuest software. Results were corrected by subtraction of values of isotype-matched controls for each data point.

2.5. Statistical analysis

All results are expressed as mean ± S.E.M. Statistical evaluation of data was performed using a one-way (ANOVA) and Bonferroni’s post-test.

3. Results

The capacity of both IFN-β and TGF-β to antagonize IFN-γ action prompted us to test their possible effect on the biological activity of the other major Th1 cytokine. IL-12, which can be monitored easily by its capacity to induce IFN-γ. In initial experiments, optimal doses of IFN-β (1000 U/ml), TGF-β (3 ng/ml) and IL-12 (10 ng/ml) were established and used throughout all further experiments.

3.1. IFN-β increases IL-12-induced IFN-γ secretion by human PBMC

As shown in Fig. 1, IFN-β by itself induced modest IFN-γ production in PBMC supernatants at 72 h of culture (101 ± 75 vs. 17 ± 10 pg/ml for control cells), which was not statistically significant (P = 0.25). As expected, IL-12 induced pronounced IFN-γ synthesis (780 ± 136 vs. 17 ± 10 pg/ml for control cells, P = 0.0035). Simultaneous addition of IFN-β and IL-12 resulted in a synergistic 86% increase in IFN-γ levels, as compared with IL-12 alone (780 ± 136 pg/ml for IL-12 vs. 1454 ± 100 pg/ml for IFN-β + IL-12), which was, statistically, highly significant (P = 0.002). This synergistic effect was also observed and even enhanced when substituting IFN-β for poly I:poly C (results not shown), a potent inducer of both IFN-α and -β, suggesting that endogenous type I IFNs might interact with IL-12 in a similar way.

3.2. TGF-β decreases IL-12-induced IFN-γ secretion by human PBMC

As shown in Fig. 2, TGF-β alone decreased slightly, but again not significantly, the low spontaneous IFN-γ secretion by control cells (8 ± 8 vs. 17 ± 10 pg/ml for control cells, P = 0.36). On the other hand, when both
cytokines were added simultaneously, TGF-β significantly decreased the IL-12-induced IFN-γ by 50% (780 ± 136 pg/ml for IL-12 vs. 393 ± 110 pg/ml for TGF-β + IL-12, P = 0.037).

Besides IFN-γ, IL-12 has been shown to induce the synthesis of TNF-α [13] and IL-10 [14], another Th1 and a Th2 cytokine, respectively. Therefore, we were interested in the possible effect of IFN-β and TGF-β on IL-12-induced TNF-α and IL-10. As shown in Fig. 3A, there was a low spontaneous production of TNF-α in control cells (44 ± 2 pg/ml), which was increased slightly by IFN-β (70 ± 5 pg/ml), and IL-12 (95 ± 16 pg/ml). When added simultaneously, IFN-β and IL-12 did not augment TNF-α in an additive manner, as compared with either cytokine alone, but remained at the level of IL-12-treated cells (99 ± 13 pg/ml for IFN-β + IL-12, vs. 95 ± 16 pg/ml for IL-12 alone). TGF-β weakly decreased both spontaneous and IL-12-induced TNF-α to 37 ± 12 and 89 ± 3 pg/ml, respectively, which was not statistically significant, P > 0.05, as was the case for all comparisons above.

Similar to TNF-α, IL-10 secretion (Fig. 3B) by control cells was low (40 ± 4 pg/ml) and, comparable to published findings [15] significantly but rather weakly induced by IFN-β (60 ± 3 pg/ml, P = 0.026). Another surprising result was the IL-12-induced decrease, instead of the expected increase [14] in IL-10 (18 ± 6 pg/ml, P = 0.026), which could be reverted by addition of IFN-β (83 ± 12 pg/ml, P = 0.033), or TGF-β (30 ± 3
pg/ml, \( P = 0.056 \). The latter cytokine by itself decreased significantly the IL-10 production, as compared with control cells (12 ± 4 pg/ml, \( P = 0.016 \)).

3.4. Adherent and non-adherent cells cooperate in their response to IFN-\( \beta \), TGF-\( \beta \) and IL-12

Since we had observed earlier that the inhibitory effects of IFN-\( \beta \) and TGF-\( \beta \) on the IFN-\( \gamma \)-induced leishmanicidal effect in human macrophages were not altered significantly by the presence or absence of lymphocytes (Van Weyenbergh & Barral-Netto, unpublished results), we sought to determine if the observed effects on IL-12 activity in total PBMC were mediated by monocytes, lymphocytes or both cell types together. As shown in Fig. 4A, the opposed effects of IFN-\( \beta \) and TGF-\( \beta \) on IL-12-induced IFN-\( \gamma \) were observed in adherent cells (Fig. 4A-A, > 80% CD14\(^+\) and CD64\(^+\) monocytes as determined by FACS analysis), as well as in non-adherent cells (Fig. 4A-NA, > 95% CD3\(^+\) or CD19\(^+\) lymphocytes as determined by FACS analysis), but absolute amounts of IFN-\( \gamma \) were lower than in total PBMC. In most donors (four out of five tested), both adherent and non-adherent cells produced almost equal amounts of IL-12-induced IFN-\( \gamma \). When comparing adhesion-separated populations with total PBMC from the same donors, their effect was additive, indicating participation of both cell types in IL-12 biological activity. In order to examine if differences in dose-response to IL-12 might account for the differential responses between the cell populations studied, we determined their IFN-\( \gamma \) production in function of IL-12 concentration. As shown in Fig. 4B, adherent cells, non-adherent cells and PBMC displayed similar dose-dependency curves for IL-12-induced IFN-\( \gamma \), with a maximum between 3 and 10 ng/ml, indicating that the differences in absolute amounts were inherent to the specific cell population and were not due to non-optimal dose or different threshold for IL-12.

On the other hand, when monocytes were purified to > 95% purity, neither IL-12 nor IFN-\( \beta \) were able to induce IFN-\( \gamma \) production (Fig. 4A-M). Moreover, no intracellular IFN-\( \gamma \) was detected by two-color flow cytometry in these stimulated monocytes (results not shown), suggesting monocytes were not the IFN-\( \gamma \)-producing cells in the adherent fraction. Another possibility is that monocytes might produce an IFN-\( \gamma \) inducing factor, such as IL-18, which is able to synergize with IL-12 for IFN-\( \gamma \) production in lymphocytes [12]. However, IFN-\( \beta \) did not induce detectable amounts of IL-18, neither in the absence nor in the presence of IL-12 (results not shown). In addition, IL-18 was unable to induce IFN-\( \gamma \) production by itself, in contrast to IFN-\( \beta \), but potentiated IL-12-induced IFN-\( \gamma \) production about ten times more efficiently than IFN-\( \beta \) (> 800% increase for IL-12 + IL-18, not shown, vs. 86% for IFN-\( \beta \) + IL-12, Fig. 1), indicating that IFN-\( \beta \) and IL-18 use different molecular pathways to synergize with IL-12.

4. Discussion

In spite of their strikingly similar antagonism on IFN-\( \gamma \) biological activity, we found that IFN-\( \beta \) and TGF-\( \beta \) diverged strongly in their effect on IL-12-induced IFN-\( \gamma \) production in human PBMC, demonstrating synergism and inhibition, respectively. These effects seemed to be limited to IFN-\( \gamma \) biosynthesis, since only modest interaction between the three cytokines was observed for TNF-\( \alpha \) or IL-10 production. Even at its maximal dose (10 ng/ml), IL-12 was unable to increase TNF-\( \alpha \) levels up to 0.1 ng/ml, in contrast to an earlier report showing IL-12 induction of TNF-\( \alpha \) [13]. This inability was not due to impaired TNF-\( \alpha \) expression in our cells, since even sub-optimal doses of LPS (< 1 ng/ml) induced pronounced TNF-\( \alpha \) expression (results
not shown). Thus it seems more likely that the observed differences can be explained by experimental conditions, i.e. in vitro versus in vivo, or species-specificity. Although the IFN-β-, IL-12- and TGF-β-induced fluctuations in IL-10 were all statistically significant \((P < 0.05)\) or close to significance \((P = 0.056)\) for TGF-β + IL-12, their physiological importance remains questionable, because of the low absolute amounts of IL-10 \(<100\;\text{pg/ml}\), which is ten-fold lower than the minimal amount \((1\;\text{ng/ml})\) of exogenous IL-10 required for significant inhibition of IFN-γ production in vitro (Van Weyenbergh et al., unpublished results). Calculating the IFN-γ/IL-10 ratio in our supernatants, resulted in a 10–40 times molar excess of IFN-γ relative to IL-10, suggesting that, in the presence of IL-12, IFN-β is able to strongly boost a Th1 type response in human PBMC.

In order to obtain clues to which cell types might mediate the effect of TGF-β and IFN-β on IL-12 action, we compared adherent cells \((>80%\;\text{monocytes})\), non-adherent cells \((>95%\;\text{lymphocytes})\), as well as \(>95%\;\text{pure monocytes with total PBMC}\;\text{(}<20%\;\text{monocytes})\). Absence of detectable IFN-γ in purified monocyte supernatants, as well as at the single cell level in flow cytometer-gated monocytes, indicated clearly that lymphocytes were the main IFN-γ-producing cells in our system, at least quantitatively. Therefore, the observed differences in IFN-γ production are not due to quantitative differences in cell numbers between the different experimental conditions, since lymphocytes present in the adherent fraction \((<20\%)\) produce ten times more IFN-γ \((1910\;\text{pg per}10^6)\) than lymphocytes in total PBMC \((195\;\text{pg per}10^6)\) on a per cell basis. We cannot exclude the possibility that adherent cells contain a sub-population of strong IFN-γ-producing lymphocytes, but it seems unlikely that these cells would be inhibited in total PBMC. Moreover, as shown in Fig. 4B, adherent cells, non-adherent cells and PBMC display the same dose-dependency for IL-12 induction of IFN-γ, arguing against different sensitivity to IL-12 in the adherent population. On the other hand, as can be deduced from Fig. 4A, both synergy with IFN-β and inhibition by TGF-β were not significantly different between adherent cells, non-adherent cells and PBMC. Taken together, these data argue for a strong cooperation between monocytes and lymphocytes for IL-12-induced IFN-γ expression, although they display equal sensitivity to the opposed effects of TGF-β and IFN-β. However, this cooperation did not seem to depend on monocyte-derived IL-18, since no endogenous IL-18 was detected in our supernatants and the effect of exogenous IL-18 was, at least quantitatively, strongly different from the effect of IFN-β or TGF-β.

As for the molecular mechanism through which TGF-β and IFN-β might interfere with the biological activity of IL-12, and more precisely the induction of the IFN-γ gene, interaction at the level of intracellular signalization might be suspected. Species-specificity in the activation of STAT4 by type I IFNs might explain the observed differences between man and mouse for regulation of Th cell development by IFN-α/β [16], but some controversies remain [6,7]. Recent reports have demonstrated the ability of TGF-β to decrease IL-12-induced IFN-γ production, but they diverge as to whether inhibition takes place at the level of IL-12-induced STAT4 activation [17], or further downstream [18]. It, thus, remains to be clarified if the differential effects of IFN-β and TGF-β observed in this and several other studies might be linked directly to STAT4 activation or inactivation.

Our findings might help explain some of the differential effects exerted by IFN-β and TGF-β in two obviously very distinct pathologies, namely multiple sclerosis (MS) and leishmaniasis, where IFN-γ has been demonstrated to play a deleterious and a protective role, respectively. IFN-β has proven to be the first drug able to slow down disease progression in multiple sclerosis patients [19,20]. However, treatment with IFN-β in vivo has been shown to augment IFN-γ producing cells in some patients during the first 2 months of treatment [21], but decreases serum IFN-γ levels afterwards [22]. This initial rise in IFN-γ might, thus, be explained by synergy between exogenous IFN-β and IL-12, the latter shown to be produced in MS lesions [23]. During the course of treatment, IFN-β might then down-regulate IFN-γ synthesis by decreasing upstream IL-12 production in antigen-presenting cells [8,9]. Hence, administration IFN-β can be considered as a two-edged sword, with the physiological outcome depending on the exact timing of its administration, dose and cellular targets. Further knowledge of this apparent ambiguity will undoubtedly lead to the improved treatment schemes.

In contrast to our findings for IFN-β, this study further confirms TGF-β as a genuine Th2 type cytokine, due to its antagonistic action towards IL-12 for IFN-γ synthesis, in addition to its role as a macrophage-deactivating factor. Therefore, induction of TGF-β by intracellular parasites might indeed be considered as a parasite escape mechanism [24,25]. Finally, it remains to be seen whether the divergent effects of IFN-β and TGF-β on IL-12 action are of physiological importance in an in vivo situation where IL-12 might be of possible therapeutic benefit, such as in leishmaniasis. Therefore, we are investigating at present if the same differential effect of IFN-β and TGF-β in vitro might be observed in patients with cutaneous leishmaniasis and whether or not their interaction with IL-12 will be modulated by the presence of Leishmania antigen or live parasites.
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