Insulin-like growth factor-I stimulates IL-10 production in human T cells

Ron Kooijman[1] and Astrid Coppens

Laboratory for Neuroendocrine Immunology, Department of Pharmacology, Medical School, Free University of Brussels (VUB), Belgium

Abstract: There is vast body of evidence that the insulin-like growth factor (IGF)-I exerts immunomodulatory effects in vitro and in vivo. In vitro studies indicate that stimulatory effects of IGF-I may be exerted through augmentation of inflammatory cytokine production. To further explore the immunomodulatory effects of IGF-I through regulation of cytokine production, we tested the in vitro effects of IGF-I on the secretion of inflammatory T helper cell type 1 (Th1) and Th2 cytokines by human peripheral blood mononuclear cells (PBMC). To this end, PBMC were stimulated with the T cell mitogen phytohemagglutinin (PHA), and cytokines in the culture media were assessed after 18, 42, 66, and 80 h of culture. We found that IGF-I stimulated the secretion of the Th2 cytokine interleukin (IL)-10 by 40–70% in PHA-stimulated PBMC. In addition, we observed a small stimulatory effect (15%) on the secretion of another Th2 cytokine IL-4. The secretion of IL-2, IL-5, IL-6, interferon-γ, and the inflammatory cytokines IL-1β, IL-8, and tumor necrosis factor α was not or was hardly affected. IL-10 secretion was also stimulated in purified T cells, and we established that IGF-I also stimulated IL-10 mRNA expression by 100–150%. The monocyte-activating bacterial cell-wall product lipopolysaccharide induced IL-10 production in PBMC, but this was not affected by IGF-I. As IL-10 predominantly exerts anti-inflammatory actions and suppresses Th1-dependent immune responses, our results indicate that IGF-I may exert inhibitory actions on inflammatory and Th1-mediated cellular immune responses through stimulation of IL-10 production in T cells. *J. Leukoc. Biol.* 76: 862–867; 2004.

Key Words: *leukocytes · cytokines · inflammation · Th1 · Th2*

INTRODUCTION

Insulin-like growth factor (IGF)-I plays a key role in embryonic and postnatal growth, exerts metabolic effects, and is involved in tissue homeostasis through regulation of cell proliferation and programmed cell death. There is ample evidence that IGF-I affects homeostasis in the immune system by enhancing lymphopoiesis, granulopoiesis, cell proliferation, and cell survival [1–3].

The effects of IGF-I on cytokine production and apoptosis of leukocytes indicate that IGF-I may also influence inflammatory reactions. For instance, IGF-I may act as a proinflammatory factor by positive effects on the onset of inflammation through stimulation of inflammatory cytokines and chemokines, such as tumor necrosis factor α (TNF-α) [4] and interleukin (IL)-8 [5], respectively. Another mechanism by which IGF-I may exert proinflammatory effects is by delaying the resolution of inflammation through inhibition of neutrophil apoptosis [5, 6]. Furthermore, IGF-I has been described to augment cell adhesion molecule expression in endothelial cells, leading to increased adhesion of monocytes [7]. There is also evidence that IGF-I can exert proinflammatory effects in vivo. It was shown that IGF-I increased the inflammatory response in rats with ischemic acute renal failure [8] and exerted protective effects in mice exposed to *Escherichia coli* [9, 10].

More insight in the regulation of immune responses by IGF-I may contribute to the design of clinical strategies for modulation of those responses through components of the IGF system. IGF-I in circulation is mostly complexed to high-affinity IGF-binding proteins (IGFBPs). Six IGFBPs have been characterized [11], and they act as carrier proteins for IGF-I involved in transport and protection against proteolytic degradation. IGFBPs are expressed in most tissues and have also been shown to modulate cellular effects of IGF-I, for instance, through blocking the binding of IGF-I to its receptor. Therefore, IGF-I, IGF-I receptor antagonists, or IGFBPs are promising therapeutic tools to modulate IGF-I-regulated processes. For instance, administration of IGF-I in combination with IGFBP3 may result in a longer half-life of IGF-I in circulation, and this treatment has been proposed as a possible therapy for patients with an acute-phase response [12, 13]. Inhibition of IGF-I action (e.g., by IGFBPs or IGF-I receptor antagonists) has also been proposed to treat certain types of cancer [14]. Furthermore, the combination of IGF-I and IGFBP-3 protects nonobese diabetic mice against type I diabetes through inhibition of β-cell apoptosis [15], and IGF-I delays the onset of experimental autoimmune encephalomyelitis (EAE) in rats [16] and

[1] Correspondence: Department of Pharmacology, Medical School, Free University of Brussels (VUB), Laarbeeklaan 103, B-1090 Brussels, Belgium. E-mail: rkooi@farc.vub.ac.be

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mice [17]. However, the mechanisms involved are not fully clear, and it has even been shown that when given after the onset of EAE, administration of IGF-I exacerbates the disease [17]. Therefore, more basic knowledge about the effects of IGF-I on immune responses may contribute to better therapeutic strategies to treat immunological disorders or diseases that have a strong immunological component or etiology.

To further explore the putative, immunomodulatory effects of IGF-I through regulation of cytokine production, we tested the effects of IGF-I on the secretion of inflammatory, T helper cell type 1 (Th1) and Th2 cytokines by human peripheral blood mononuclear cells (PBMC). In this paper, we show that IGF-I stimulates IL-10 mRNA expression and IL-10 secretion in phytohemagglutinin (PHA)-stimulated peripheral blood T cells, whereas the production of other tested cytokines was not or was hardly affected.

MATERIALS AND METHODS

Reagents

Recombinant human IGF-I was kindly provided by Lilly Research Laboratories (Indianapolis, IN). Contamination with endotoxin was not detectable using the limulus amebocyte lysate assay (detection limit 1.25 pg/μg; BioWhittaker, Walkersville, MD). PHA-15 was from Murex Diagnostics (Dartford, UK). Lipopolysaccharides (LPS), from Salmonella typhosa, 2 aminoethyl-isothiouronium bromide (AET), and bovine serum albumin (BSA), were purchased from Sigma (Belgium), and type-1 IGF receptor antibody αIR3 was obtained from Oncogene (Darmstadt, Germany). RPMI, penicillin, and streptomycin were obtained from Invitrogen (Merelbeke, Belgium).

Cell preparation and cell culture

Human PBMC were purified from heparinized venous blood drawn from healthy donors between 20 and 60 years of age. Informed consent was obtained from all blood donors, and the local ethical committee approved the research protocol. Mononuclear cells were purified by centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway), and T cells were isolated from PBMC by rosetting with sheep erythrocytes that were pretreated with AET. The contamination of T cell preparations with other cells, as assessed by flow cytometry, was less than 10%, and cell viability as assessed by trypan blue exclusion was always higher than 95%. Freshly isolated cells were suspended at a density of 10^6 cells/ml in serum-free medium (RPMI 1640 with glutamax-I, supplemented with 0.02% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin) and cultured in 5 ml polystyrene Falcon tubes (Becton Dickinson, Erembodegem, Belgium) in a humidified 5% CO_2 atmosphere at 37°C. After culture, cells were separated from the culture medium by centrifugation for 10 min at 400 *g* at room temperature. To study the effects of IGF-I on cytokine production, PBMC were cultured in the absence or presence of IGF-I. In all experiments, IGF-I and 2 μg/ml PHA were added directly at the start of the culture period.

Cytokine assays

Culture media were frozen and stored at –20°C until use. Cytokine levels were quantified by enzyme-linked immunosorbent assays (ELISAs) using commercial antibody pairs (Cytosets™) from Biosource International (Nivelles, Belgium). The levels of IL-1β, IL-6, TNF-α, or IL-8 were determined as described earlier [5]. ELISAs for IL-2, IL-4, IL-5, IL-10, and interferon-γ (IFN-γ) levels were carried out according to the manufacturer's protocol using the following antibody concentrations: 1.0 μg/ml IL-2 coating antibody (clone 419A7A3); 0.5 μg/ml IL-2 detection antibody (297C1662); 1.0 μg/ml IL-4 coating antibody (860A4B3); 0.125 μg/ml IL-4 detection antibody (860F10H12); 1.0 μg/ml IL-5 coating antibody (JES1-39D10); 1.0 μg/ml IL-5 detection antibody (JES1-5A10); 1.0 μg/ml IL-10 coating antibody (945A5D11); 0.2 μg/ml IL-10 detection antibody (945A5A10); 1.0 μg/ml IFN-γ coating antibody (350B1066); 0.2 μg/ml IFN-γ detection antibody (67F12A8). The sensitivities of the ELISAs for IL-2, IL-4, IL-5, IL-10, and IFN-γ were 8, 8, 8, 8, and 20 pg/ml, respectively.

Real-time polymerase chain reaction (PCR)

Total RNA was isolated by extraction with Trizol using the standard procedure supplied by the manufacturer (Invitrogen). Reversed transcription of 250 ng total RNA was performed using a reverse transcription kit (Applied Biosystems, Lennik, Belgium) containing random hexamers. The real-time PCR amplification of cDNA (undiluted) from PBMC was performed on an ABI prism 7700 sequence detector (Perkin Elmer, Boston, MA) using an IL-10 assay-on-demand kit from Applied Biosystems. The results of a series of standards prepared by successive dilutions (30–2500 ng) of total RNA and plotted against the logarithm of the concentration were used to estimate the relative amount of specific mRNA initially present in the various samples. The relative IL-10 mRNA levels compared with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) controls are shown.

Statistical analysis

Statistical comparisons were made using the one-sample Student's *t*-test to determine whether values expressed as a percentage of controls differ significantly from 100% (see Tables 1 and 2 and Fig. 1A) and the unpaired two-tailed Student's *t*-test when means of two groups were compared (see Fig. 2). A repeated measures ANOVA with a Dunnett post-test was used when means of more than two groups were compared (Fig. 1B). A probability value less than 0.05 was considered significant.

RESULTS

Effects of IGF-I on cytokine secretion by PHA-activated PBMC

To address the putative actions of IGF-I on different components of the immune system through modification of cytokine production, we investigated the effects of IGF-I on the secretion of inflammatory, Th1 and Th2 cytokines. Cytokine levels in the culture medium of PHA-activated PBMC from six different donors were measured at 18, 42, 66, and 80 h after stimulation with PHA in the absence or presence of 50 ng/ml IGF-I (**Table 1**). To test the null hypothesis that IGF-I does not influence the secretion of a certain cytokine at any tested time-point, we performed a one-sample, two-tailed Student's *t*-test with Bonferroni correction for multiple comparisons. We found that IL-10 levels in the culture media were significantly increased by 62%, 67%, and 44% after 42, 66, and 80 h, respectively. The concentration of another anti-inflammatory Th2 cytokine, IL-4, was also significantly increased, albeit to a much lesser extent than IL-10. In addition, a small but significant decrease in the concentration of the Th1 cytokine IFN-γ was observed after 4 days of culture. Although PHA is a T cell-activating lectin, we also measured the secretion of monocyte-derived inflammatory cytokines, which might be induced by T cell-derived cytokines. Small but significant effects of IGF-I were found on the secretion of inflammatory cytokines such as TNF-α, IL-1β, and IL-8. However, the effects on TNF-α and IL-8 were markedly smaller than the effects observed in the absence of a stimulus or in the presence of the monocyte-activating stimulus LPS [4, 5]. To assess the effects of IGF-I on IL-10 secretion in LPS-stimulated cells, we cultured PBMC in the absence and the presence of 5.0 μg/ml LPS for 1–3 days. It appeared that IL-10 was not detected in the conditioned media of PBMC cultures in the absence of LPS and

TABLE 1. Effects of IGF-I on Cytokine Secretion by PHA-Activated PBMC

	18 h	42 h	66 h	80 h
IL-10	120.2 ± 8.4[a]	161.8 ± 12.9*	167.2 ± 20.5**	143.9 ± 5.8*
IL-4	116.5 ± 5.0**	115.1 ± 5.6**	107.7 ± 3.5	100.2 ± 1.7
IL-5	104.1 ± 3.1	109.8 ± 8.2	103.4 ± 4.6	99.8 ± 4.3
IL-6	90.0 ± 7.6	108.8 ± 7.2	92.7 ± 5.6	111.9 ± 3.5
IL-2	98.2 ± 5.6	105.6 ± 4.7	98.9 ± 1.3	100.9 ± 0.6
IFN-γ	108.7 ± 5.8	111.9 ± 7.1	97.9 ± 7.6	89.3 ± 1.6*
IL-1β	106.4 ± 5.7	111.4 ± 9.3	103.7 ± 1.9	111.5 ± 2.7**
TNF-α	106.4 ± 2.0**	105.3 ± 2.7	104.7 ± 1.1*	104.9 ± 3.4
IL-8	102.8 ± 1.5	105.8 ± 1.6**	110.3 ± 4.4	106.2 ± 1.1*

[a] Relative cytokine levels in the conditioned media of PBMC cultured in the presence of 50 ng/ml IGF-I as a percentage of controls (in the absence of IGF-I). The data represent the mean values ± SEM from six different donors. The mean cytokine levels in the culture media of PHA-stimulated cells from six donors in the absence of IGF-I were calculated at each time-point. During the culture period, these values varied among the following levels: IL-10: 30–77 pg/ml; IL-4: 10–17 pg/ml; IL-5: 39–74 pg/ml; IL-6: 6288–8894 pg/ml; IL-2: 75–267 pg/ml; IFN-γ: 12,016–46,724 pg/ml; IL-1β: 981–2070 pg/ml; TNF-α: 4975–7269 pg/ml; IL-8: 33,463–72,294 pg/ml. * $P < 0.01$. ** Significantly different ($P<0.05$) from 100%, as assessed by a one-sample, two-tailed Student's t-test with Bonferroni correction for multiple comparisons.

that IGF-I did not affect the secretion of IL-10 by LPS-activated cells (**Table 2**).

IGF-I-induced IL-10 production

To establish the role of IGF-I in the regulation of IL-10 mRNA levels in PHA-stimulated PBMC, we assessed the effects of IGF-I on IL-10/GAPDH mRNA ratios at 18, 42, and 66 h of culture in six independent experiments. We calculated the mean stimulation by IGF-I of IL-10/GAPDH ratios, as the basal ratios in PBMC from different donors were highly variable. **Figure 1A** shows that IGF-I significantly enhanced IL-10 mRNA expression by 100–150% in PHA-activated PBMC within 18 h. The stimulating effects of IGF-I at 42 and 66 h were not significant. IL-10 levels in conditioned media were undetectable when the cells were not stimulated with PHA in the absence or in the presence of IGF-I (data not shown). As shown in Figure 1B, PHA induced IL-10 production within the first 18 h of the culture period. Maximal levels of IL-10 in the conditioned media were reached after 42 h. IGF-I (50 and 300 ng/ml) induced a strong significant increase in IL-10 levels at all time-points. The strongest effects of IGF-I on IL-10 levels in the conditioned media were detected at 44 and 66 h.

IGF-I stimulates IL-10 secretion by T cells

As IL-10 can be produced by monocytes, B cells, and T cells, we tested the effects of IGF-I on purified T cells and on the non-T cell fraction of PBMC. **Figure 2** shows a representative experiment in which IL-10 secretion by PHA-activated T cells is significantly enhanced by IGF-I. Notably, PHA did not induce detectable levels of IL-10 secretion by non-T cells in any of the four experiments. The mean increase in IGF-I secretion by T cells was 40 ± 7% and significantly different from 0 ($P<0.05$; n=4). This effect is slightly smaller that the effect on PBMC (55±4%; $P<0.01$). This phenomenon and the reduction in IL-10-producing ability after T cell isolation were not a result of the removal of non-T cells, as reconstitution of purified T cells with non-T cells had no effect on IL-10. In contrast, we found that a lag period between isolation of PBMC and stimulation with PHA negatively influenced IL-10 production and stimulation by IGF-I (data not shown).

DISCUSSION

There is ample evidence that IGF-I exerts immunomodulatory effects in vitro and in vivo. We and others have shown that in vitro, IGF-I stimulates the secretion of the several cytokines. In the present study, we investigated the effects of IGF-I on the production of Th1 cytokines, Th2 cytokines, and monocyte-derived inflammatory cytokines by human PBMC in the presence of the polyclonal, T cell-activating lectin PHA. A strong, significant stimulating effect of IGF-I was observed on mRNA expression and secretion of IL-10, whereas the secretion of other cytokines was not or was marginally affected.

Tu et al. [18] measured cytokine levels in the conditioned media of PHA-stimulated PBMC after a 3-day culture period. In agreement with our observations, they found no effects of IGF-I on the levels of IL-2 and IL-4. However, this group also found that IGF-I enhances basal and PHA-stimulated IL-6 production in PBMC. Indeed, in a previous study, we observed a significant but smaller (29%) stimulation of basal IL-6 production after 18 h of culture [5]. The absence of any effect of IGF-I on IL-6 secretion in PHA-stimulated cells in this study may be a result of a relatively strong stimulatory effect of PHA on IL-6 production. We used 2 μg/ml PHA instead of 1 μg/ml,

TABLE 2. IGF-I Effects on IL-10 Secretion by LPS-Activated PBMC

	18 h	42 h	66 h
5.0 μg/ml LPS	114.7 ± 13.0[a]	92.2 ± 2.2*	88.4 ± 7.4

[a] Relative cytokine levels in the conditioned media of PBMC cultured in the presence of 50 ng/ml IGF-I as a percentage of controls (in the absence of IGF-I). The data represent the mean values ± SEM from five to seven different donors. * Significantly different ($P<0.05$) from 100%, as assessed by a one-sample, two-tailed Student's t-test with Bonferroni correction for multiple comparisons.

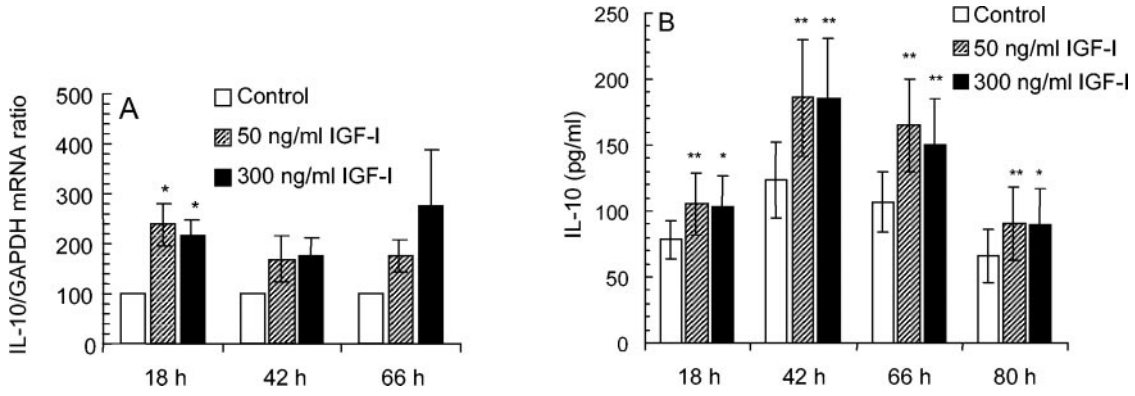

Fig. 1. (A) Effects of IGF-I on relative IL-10 mRNA levels in human PBMC. PHA-stimulated PBMC were cultured in quadruplicate for 1, 2, or 3 days in serum-free medium with or without IGF-I. The histogram depicts the IL-10/GAPDH mRNA ratios in the presence of 50 or 300 ng/ml IGF-I compared with control values in the absence of IGF-I (100%). Mean values ± SEM of six independent experiments are shown. *, Significantly different ($P<0.05$) from 100%, as assessed by the one-sample, two-tailed Student's *t*-test with Bonferroni correction for multiple comparisons. (B) Kinetics of IGF-I effects on IL-10 accumulation in conditioned media. Cells were cultured for 1–4 days as described above. IL-10 was not detectable in conditioned media of cells cultured in the absence of PHA. The data represent the mean values ± SEM of nine independent experiments. *, $P < 0.05$; **, $P < 0.01$.

and the IL-6 levels in the presence of PHA alone were at least 10 times higher as compared with the levels found by Tu et al. [18]. Thus, the effects of IGF-I on cytokine secretion may depend on the strength of the T cell stimulus. The above may also explain that these investigators found a small, stimulatory effect of IGF-I on IFN-γ secretion by PBMC. As the effect on IFN-γ was stronger in neonatal, mononuclear cells, the maturity of the immune system may also influence the effects of IGF-I on cytokine secretion. The finding that basal and PHA-stimulated IL-6 secretion in neonatal mononuclear cells is augmented by IGF-I also indicates a role for IGF-I in modulation of cytokine production in neonatal leukocytes [18]. In addition, reduction of IGF-I receptor expression using antisense oligonucleotide technology in neonatal mononuclear cells resulted in decreased mRNA levels for IFN-γ, IL-2, and IL-4 [19]. Moreover, interactions between different PBMC subpopulations may also define the outcome of treatment with IGF-I on cytokine secretion. Two studies showed that IGF-I enhances PHA-stimulated IL-2 secretion in purified T cells [20, 21], whereas this effect was not observed when PBMC were treated with IGF-I (Table 1) [18].

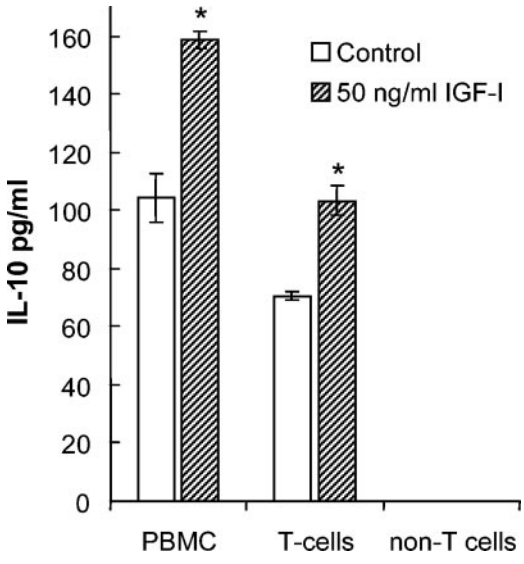

Fig. 2. IGF-I effects on IL-10 secretion by PBMC, purified T cells, and non-T cells. Cells were cultured for 42 h as described in Figure 1. The data represent the mean values ± SEM of quadruplicate incubations. *, $P < 0.001$. The results are representative of four independent experiments. The average stimulation of IL-10 secretion by 50 ng/ml IGF-I in PBMC and T cells was 55 ± 4% (n=4; $P<0.01$) and 40 ± 7% (n=4; $P<0.05$), respectively. IL-10 concentrations in the conditioned media from non-T cells were always below the detection level.

It is remarkable that the secretion of proinflammatory cytokines was only marginally increased, whereas other studies showed much stronger effects of IGF-I on inflammatory cytokine secretion. For instance, a 120% increase in basal TNF-α production and a stimulation of mRNA expression were observed when purified human monocytes were incubated with IGF-I [4]. In a previous study, we found that IGF-I increased TNF-α secretion by LPS-stimulated human PBMC by 24%. We also reported that basal and LPS-stimulated IL-8 secretion in human PBMC was enhanced by 48% and 43%, respectively [5]. Here, we show that IGF-I stimulates IL-10 secretion by PHA-activated T cells, whereas IGF-I did not influence IL-10 secretion in LPS-activated PBMC and that IL-10 was not detected in conditioned media of unstimulated cells (Table 2). Taken together, these results indicate that the effect of IGF-I on cytokine production depends on the nature of the stimulus or the cell type involved. In the presence of LPS, a monocyte-activating bacterial cell-wall component, IGF-I predominantly enhances the secretion of inflammatory cytokines, whereas in PHA-activated T cells, IGF-I predominantly stimulates the secretion of IL-10. The most important effect of IL-10 is to impair inflammatory reactions [22]. IL-10 is well recognized as an important negative regulator of proinflammatory gene expression in monocytes. It inhibits the production of TNF-α, IL-1β, IL-6, IL-12, and many chemokines including IL-8. These effects are in accordance with the finding that IL-10-deficient mice exhibit high levels of inflammatory cytokines and develop chronic inflammatory diseases [23]. Therefore, our results implicate that IGF-I has the potential to exert anti-inflammatory actions through stimulation of IL-10 production in activated T cells. However, it is well known that the clas-

sification of cytokines in inflammatory and anti-inflammatory cytokines is too simplistic, and one should take into account that IL-10 has also been reported to exert proinflammatory effects [24]. The in vivo effects of IGF-I on IL-10 and inflammatory responses are currently being investigated by us in a mouse model for peritonitis. In addition to anti-inflammatory cytokines, IL-10 also inhibits the production of Th1 cytokines [22] and as a consequence, the development of Th1 cells. As such, IGF-I may induce a shift from the production of Th1 cytokines to Th2 cytokines, leading to a diminution of cellular immune responses and a stimulation of antibody-mediated responses. Furthermore, IL-10 directly enhances the survival, proliferation, and differentiation of B cells [22]. The above indicates that IGF-I may stimulate antibody production in vivo [25] through stimulation of IL-10 production.

Several groups have proposed autocrine or paracrine functions for IGF-I in the immune system. As IGF-I is predominantly produced by macrophages [26], the possibility exists that macrophage-derived IGF-I serves, for instance, during antigen presentation, as a paracrine factor inducing IL-10 production in T cells. The presence of leukocyte-derived IGF-I in the cultures could influence IL-10 production in our experiments and lead to an underestimation of IGF-I effects. However, previous experiments addressing lymphocyte proliferation under similar serum-free conditions showed that low levels of IGF-I are effective and that the inhibiting IGFBP-1 does not affect proliferation in the absence of exogenous IGF-I [27]. These results argue against possible effects of leukocyte-derived IGF-I in our culture system.

The relation between IGF-I and the immune system is not only of academic interest. More insight in this relation may contribute to the design of clinical strategies for modulation of immune responses through components of the IGF system, e.g., IGF-I, IGFBPs, or IGF-I-neutralizing antibodies. Administration of IGF-I in combination with its predominant binding protein IGFBP-3 to severely burned children induced a raise in plasma IL-10 levels and a reduction of IL-6 and TNF-α levels [28]. The decreased ratio of pro- to anti-inflammatory cytokines may protect patients with an acute-phase response from multiorgan failure. In another study, IGF-I was shown to augment plasma IL-10 concentrations in rats [29]. This effect went along with a protective effect on induction of pancreatitis by caerulein. Although IL-10 was also shown to exert beneficial effects, a causal relation between IL-10 and the protective effects of IGF-I was not established.

IGF-I has also been proposed as a therapeutic agent in neurodegenerative diseases, as it is a potent stimulator of oligodendrocyte development and myelination. EAE is an animal model for multiple sclerosis in which demyelination occurs as a result of a Th1-mediated autoimmune response. Indeed, under certain conditions, IGF-I protects against the development of EAE in rats and mice [16, 17]. However, in addition to direct stimulation of remyelination, immunomodulatory actions of IGF-I may be responsible for the clinical effects in EAE models. As IL-10 has been reported to protect against EAE and other Th1 autoimmune responses such as type I diabetes [22], it is tempting to speculate that IL-10 may be involved in the protective effects of IGF-I. It is important that with regard to the pleiotropic effects of IGF-I and the differential effects of IGF-I treatment at different stages of the EAE [17], further studies about the in vivo immunomodulatory actions of IGF-I are required and may be performed using IGF-I- and IL-10 knockout mice.

Inhibition of IGF-I receptor activation by components of the IGF system has also been proposed to inhibit the growth of cancer cells, as IGF-I stimulates the proliferation and survival of many types of cancer cells. It was recently shown that an antibody against the IGF-I receptor inhibited the in vivo growth of renal, breast, and pancreatic tumors [30]. A concomitant inhibition of IL-10 production may be advantageous, as IL-10 has been indicated to suppress the antitumor immune response [31]. In addition, there are several lines of evidence that IL-10 overexpression in different types of cancer may contribute to tumor development, and IL-10 has been implicated as a tumor cell growth factor in B cell lymphoma [32], melanomas [33], and myeloma cells [34].

In conclusion, although most reports revealed immunostimulatory effects of IGF-I, our results indicate that IGF-I also has the potential to exert anti-inflammatory actions via stimulation of IL-10 production in activated T cells. Through stimulation of this Th2 cytokine, IGF-I may also induce a shift from Th1 to Th2 responses, leading to, for instance, suppression of cellular immunity and enhanced antibody-mediated immune responses. These possibilities should be taken into account when considering therapeutic strategies targeting the IGF system to treat immune disorders or diseases with a strong immunological component.

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