Biochemistry
—and—
Molecular Biology

Regulation of IgE and Type II IgE receptor expression by insulin-like growth factor-1: Role of STAT6 and NF-κB.

Hyun-Ja Koh, Hyun-Hee Park and Choong-Eun Lee*

Department of Biological Science and Institute for Basic Science, SungKyunKwan University, Suwon 440-746, Korea

Interleukin-4(IL-4) is known to be a major cytokine regulating immunoglobulin E(IgE) response by the induction of IgE production and type II IgE receptor(IgER II: CD23) expression. Recently, however, the role of neuroendocrine factors has been implicated in modulating the IgE response. Among various neuroendocrine growth factors, we investigated the effects of the insulin-like growth factor-1(IGF-1) since IL-4 and IGF-1 share common intracellular signaling molecules, such as the insulin receptor substrate-1/2(IRS-1/2) to induce a specific cellular response. In the human peripheral blood mononuclear cell (PBMC) cultures, IGF-1 was capable of inducing a substantial level of IgE production in a dose-dependent manner. It also noticeably upregulated the IL-4-induced or IL-4 plus anti-CD40-induced IgE production. Similarly, the IGF-1-induced IgE production was enhanced by IL-4 or anti-CD40 in an additive manner, which became saturated at high concentrations of IGF-1. Although IGF-1 alone did not induce IgER II (CD23) expression, it augmented the IL-4-induced surface CD23 expression in a manner similar to the action of anti-CD40. These results imply that IGF-1 is likely to utilize common signaling pathways with IL-4 and anti-CD40 to induce IgE and IgER II expression. In support of this notion, we observed that IGF-1 enhanced the IL-4-induced signal transducers and activators of transcription 6(STAT6) activation and independently induced NF-κB activation. Both of these bind to the IgE(C) or IgER II (CD23) promoters. Together, our data suggest that IL-4 and IGF-1 work cooperatively to activate STAT6 and NF-κB. This leads to the subsequent binding of these transcription factors to the Cε and CD23 promoters to enhance the expression of IgE and IgER II. The observed differential ability of IGF-1 on the induction of IgE vs. IgER II is discussed based on the different structure of the two promoters.

*To whom correspondence should be addressed.
Tel: 82-31-290-7006/7016; Fax: 82-31-290-7006/7015
E-mail: celee@yurim.skku.ac.kr

Introduction

Recently, the neuroendocrine modulation of the immune response has become an important concept in the field of immunophysiology. This is substantiated by a number of reports that certain endocrine hormones, neuropeptides, and growth factors can affect immune cell activation and immunoglobulin(Ig) production *in vivo* and *in vitro* (Morell, 1995; Wilder, 1995). While the production of IgM, IgG and its subtypes, or IgA is regulated by various cytokines in a somewhat redundant manner, the production of IgE is rather strictly regulated by specific cytokines (Finkelman *et al.*, 1990; Stavnezer, 1996). In fact, it has been accepted that IgE production by B cell is mainly induced by interleukin-4(IL-4), a Th2 cell-derived cytokine in the presence of CD40 co-stimulation by CD40 ligand or anti-CD40 treatment (Gascan *et al.*, 1991; Warren and Berton, 1995). In addition to IgE production, the induction of the IgER II (the low affinity IgE receptor; FcεRII) expression is an *important factor in the* regulation of an allergic response. The role of IgER II has been suggested for IgE-dependent antigen focusing, antigen uptake, and presentation to T cells to subsequently stimulate IL-4 production by T helper type 2(Th2) cells (Kehry and Yamashita, 1989; Mudde *et al.*, 1995). In fact, stimulation of B cells through the IL-4 receptor (IL-4R) and CD40 can induce the coordinated upregulation of IgE production and IgER II expression (Jabara *et al.*, 1990; Kim *et al.*, 1997).

In recent years various neuroendocrine factors, such as insulin, insulin-like growth factor-1(IGF-1), somatostatin, steroid, vasoactive intestinal peptide, and substance P, have been implicated in the potential regulation of IgE production (Jabara *et al.*, 1991; Kimata *et al.*, 1992; Kimata *et al.*, 1994; Carucci *et al.*, 1995; Wang *et al.*, 1996). Insulin and IGF-1 are of particular interest because they share with the IL-4R or

CD40 system, common intracellular signaling molecules such as insulin receptor substrate-1/2 (IRS-1/2) or nuclear factor-κ B (NF-κB), respectively (Wang et al., 1993; Berberich et al., 1994; Jakson et al., 1998). In an effort to promote the understanding on the neuroendocrine-immune interaction, the present study was conducted in order to investigate the regulation of IgE production and IgER II expression by cytokines and neuroendocrine factors. In this article we report the regulatory effects of IGF-1 on the IgE and IgER II expression. IGF-1 enhances the IL-4-induced IgE and IgER II expression in human primary lymphocytes. This process is likely to involve the modulation of signal transducers and activators of transcription 6(STAT6) and NF-κB, two major signaling molecules which have been implicated for the IL-4R and CD40 signal transduction system to activate IgE and IgER II genes.

Materials and Methods

Culture and stimulation of immune cells   Human primary immune cells were obtained by isolation from freshly excised tonsils or fresh peripheral blood from normal donors using Ficoll-Hypaque (d = 1.077) through density gradient centrifugation. When necessary, non-adherent mononuclear cells (MNCs) were obtained by removing adherent cells from total mononuclear cells using plate adherence by incubating cells on a 100 mm plate at 37°C for 1 h. Unless otherwise indicated, cells were cultured or treated with IL-4, an agonistic anti-CD40 antibody (G28-5), or IGF-1 in complete RPMI media at 37°C with 5% CO_2.

IgE ELISA   A two-site ELISA for the human IgE detection system has been developed using two monoclonal anti-human IgE Abs (G7-18 and biotin-conjugated G-76, Pharmingen, San Diego, USA). To induce IgE production, peripheral blood mononuclear cells (PBMCs) were seeded on round-bottomed 96-well plates at 3×10^5 cells/0.2 ml/well. IL-4 (R & D systems Inc., Mckinley, USA), agonistic anti-CD40 (G28-5, Immunotech, westbrook, USA), or IGF-1 (Sigma, St. Louis, USA) was added to cells and cultured for 14 days. Then ELISA was performed as follows: Flat-bottomed 96-well plates were coated with G7-18 anti-human IgE mAb that were diluted in the assay buffer (PBS containing 0.05% BSA). After washing 4 times with a washing buffer (PBS containing 0.05% Tween 20), the plates were incubated with a blocking buffer (PBS containing 1% BSA) for 1 h at room temperature. The IgE standard (diluted human myeloma serum, Pharmingen, San Diego, USA) or culture supernatants were added to the prepared plates. The plates were then incubated overnight. The plates were further incubated with biotin-conjugated G7-26 mAbs for 2 h, and then with streptavidin-horseradish peroxidase (1 : 1000, Pharmingen) for 1 h at 37°C. Color was developed using O-phenylenediamine as a substrate for 30 min, after which a stop buffer (3 M H_2SO_4) was added (Park et al. b, 1998). O.D. was determined at 490 nm using an ELISA reader (ELX800, Bio-tek).

Flow Cytometric analysis of IgER II (CD23)   Surface expression of FcεRII/CD23 on tonsillar mononuclear cells was

analyzed by FACS Calibur (Becton Dickinson, Mountain View, USA) using anti-human CD23 mAb conjugated with phycoerythrine (PE, Becton-Dickinson, Mountain View, USA) after 24 h exposure to cytokines, as described previously (Kim et al., 1998).

Electrophoretic mobility shift assay(EMSA)   Nuclear extract preparation for EMSA was done after simulation of cells with cytokines for 30 min in a serum-free media. STAT6-binding GAS (gamma activation site) oligomer (Cε/GAS or CD23/GAS), NF-κB site oligomer (Kim et al., 1997), or oligomers containing both NF-κB and STAT6 sites derived from the Cε and CD23 promoter (Koler and Rieber, 1993; Thienes et al., 1996) were synthesized and labeled with [α-^{32}P] dCTP by Klenow (Min and Paik, 1998). The nuclear extracts (5~10 μg) were incubated with the labeled oligomer in the binding buffer for 20 min at room temperature. For antibody supershift assays, polyclonal anti-STAT6 or NF-κB(p65) Abs (Upstate Biotechnologies Inc., Lake Placid, USA) were incubated with the cell extracts for 30 min prior to the oligomer addition. The mobility shift of the oligomer was then analyzed by 5% PAGE in a 0.5×Tris/borate/EDTA(TBE) buffer as previously described (Park et al. a, 1998).

Results

Induction of IgE production by IGF-1 in human PBMCs

As a part of our on-going study on the regulation of the IgE response in the context of a neuroendocrine-immune network, we wanted to investigate the effects of a number of neuroendocrine growth factors on the IgE production in human PBMCs. For this, we first developed a sensitive human IgE Elisa system employing two monoclonal anti-human IgE antibodies which recognize the distinct epitopes and signal-amplifying system using avidin-biotin. The lower detection limit of this IgE ELISA system was 1 pg/ml.

In order to validate our IgE production-detection system, we first examined the effect of IL-4 and agonistic anti-CD40 antibody (G28-5) on the IgE production in human PBMC cultures. As expected, IL-4 induced IgE in a dose-dependent manner, and the addition of anti-CD40 on the IL-4-treated culture, significantly enhanced the IL-4-induced IgE (Fig 1). As reported previously (Gascan et al., 1991; Warren and Burton, 1995), the effect of CD40 cross-linking was synergistic in that the co-treatment of cells with an anti-CD40 antibody induced a higher level of IgE than the maximum levels induced by IL-4 alone (Fig 1). CD40 ligation alone, however, did not induce significant levels of IgE over the wide concentration range (data not shown).

We then tested various neuroendocrine growth factors for their ability to modulate the IL-4- or IL-4 plus anti-CD40-induced IgE production. Among a number of factors examined, IGF-1 exhibited the most prominent effect. IGF-1 at 250 ng/ml not only enhanced the IL-4- or IL-4 plus anti-CD40-induced IgE production, but also independently induced a significant level of IgE in our culture system (Fig 2-A). In fact, the effect of IGF-1 on IgE induction was IGF-1
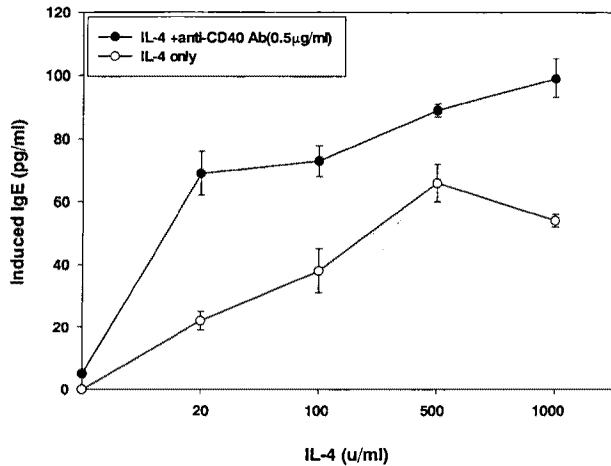
Fig. 1. Synergistic effect of anti-CD40 on the IL-4-induced IgE production in human PBMCs. Normal human PBMCs were cultured in the presence of media alone, or varying concentrations of IL-4 with or without the addition of anti-CD40 (0.5 μg/ml) for 14 days. Culture supernatants were assayed for IgE production by ELISA, as described in Materials and Methods. Induced IgE was calculated as the IgE level in the IL-4- or anti-CD40- treated culture minus (-) the IgE level in untreated culture. Each data point represents an average value of triplicate samples with a standard deviation shown.

dose-dependent in the concentration range of 250 to 2000 ng/ml (Fig 2-B). We also observed that the addition of IL-4 or anti-CD40, substantially upregulated the IGF-1-induced effect in the low concentration range of IGF-1. These effects became saturated at high concentrations of IGF-1 over 500 ng/ml (Fig 2-B, Fig 2-C).

The results suggest that signaling pathways utilized by IGF-1 to induce IgE share common intracellular components with the pathways activated upon IL-4 or anti-CD40 stimulation.

Upregulation of the IL-4-induced IgER II (CD23) by IGF-1 In addition to IgE, IgER II (FcεRII: CD23) has been strongly implicated in the regulation of allergic responses through participating in the IgE-mediated allergen uptake, processing, and presentation by B cells and monocytes (Kehry and Yamashita, 1989; Mudde *et al.*, 1995), as well as directly modulating IgE production by B cells (Fujiwara *et al.*, 1994; Sarfati and Delespesse, 1998). The concerted action of IgE and IgER II in modulating allergy has been supported by the findings that expressions of these two molecules are regulated in a coordinated manner (Lee *et al.*, 1995). That is, stimuli that increase IgE production (such as IL-4, IL-13, or anti-CD40) upregulate the IgER II expression (Defrance *et al.* 1987; Gauchat *et al.*, 1990; Punnonen *et al.*, 1993). Also, stimuli that suppress IgE production (such as IFN-γ or IFN-α) have been shown to down-regulate the IL-4-induced IgER II expression (Xu and Rothman, 1993; Lee *et al.*, 1993). Thus, having observed the significant IgE-inducing ability of IGF-1, we were interested in examining its ability to regulate the

IgER II expression in human lymphocyte cultures.

Using tonsillar mononuclear cells, we performed an analysis of the surface CD23 expression by flow cytometry. As shown in Fig 3, IL-4 effectively induced the CD23 expression, which was enhanced by the addition of an anti-CD40 antibody (ΔMFI: 3.0 vs. 160.4 vs. 185.6). While IGF-1 itself had no effect (ΔMFI: 3.0 vs. 5.5), it substantially potentiated the IL-4-induced CD23 level with a greater magnitude than anti-CD40 stimulation did (ΔMFI: 214.3 vs. 160.4). Although IGF-1 or anti-CD40, each or together, had no CD23-inducing effect (ΔMFI: 3.0 vs. 5.5 vs. 3.5 vs. 3.3), the co-addition of IGF-1 and anti-CD40 to the IL-4-treated culture resulted in a further increase of the IL-4-induced CD23 level (ΔMFI: 263.5 and 160.4). When the effect of IGF-1 alone on the CD23 induction was tested in a broader concentration range, there was still no induction noted (Fig 4-A). Importantly, the addition of IL-4 to the IGF-1-treated culture revealed that the potentiating effect of IGF-1 on the IL-4-induced CD23 was optimal between 100 and 250 ng/ml. At over 250 ng/ml, the enhancing effect of IGF-1 on the IL-4-induced CD23 expression seemed to be saturated (Fig 4-A).

At 250 ng/ml, IGF-1 not only worked additively with IL-4 to enhance IgE production, as in Fig 2-A and 2-B, but also substantially upregulated the IL-4-induced CD23 level in a broad concentration range of IL-4 reaching to a level above the maximum level induced by IL-4 (Fig 4-B). This profile closely resembled the CD23 induction pattern that was obtained by co-treatment of IL-4 and anti-CD40 (Fig 4-C). This indicates that, as in the case of anti-CD40 co-stimulation, IGF-1 acts synergistically with IL-4 to upregulate the CD23 expression. These results suggest that, although the IGF-1-mediated signal alone was not sufficient to induce CD23 expression, IGF-1 augments the IL-4-induced CD23 expression via signaling pathways distinct from those utilized by IL-4.

Role of STAT6 and NF-κB in the IGF-1 modulation of IgE and IgER II It has been reported that IL-4 induces IgE production and IgER II expression via transcriptional activation, and the synergistic effect of anti-CD40 stimulation on these IL-4-induced responses is also thought to be mediated by an increase in transcription (Fenghao *et al.*, 1995; Iciek *et al.*, 1997).

In this regard, we previously reported that the synergism between IL-4 and CD40 ligation on CD23 induction is exerted through the action of distinct transcriptional factors, IL-4-induced STAT6 and anti-CD40-induced NF-κ B via protein tyrosine kinase-dependent pathways (Kim *et al.*, 1997). STAT6 is the unique IL-4 STAT activated via tyrosine phosphorylation by IL-4R-associated Jak1/Jak3, and subsequently translocates to the nucleus to bind STAT-binding elements known as GAS (Fanghao *et al.*, 1995). NF-κB is reported to be activated by signals generated by activated receptors of tumor necrosis factor receptor (TNFR) family, including CD40 possibly via a TNF receptor-associated factor
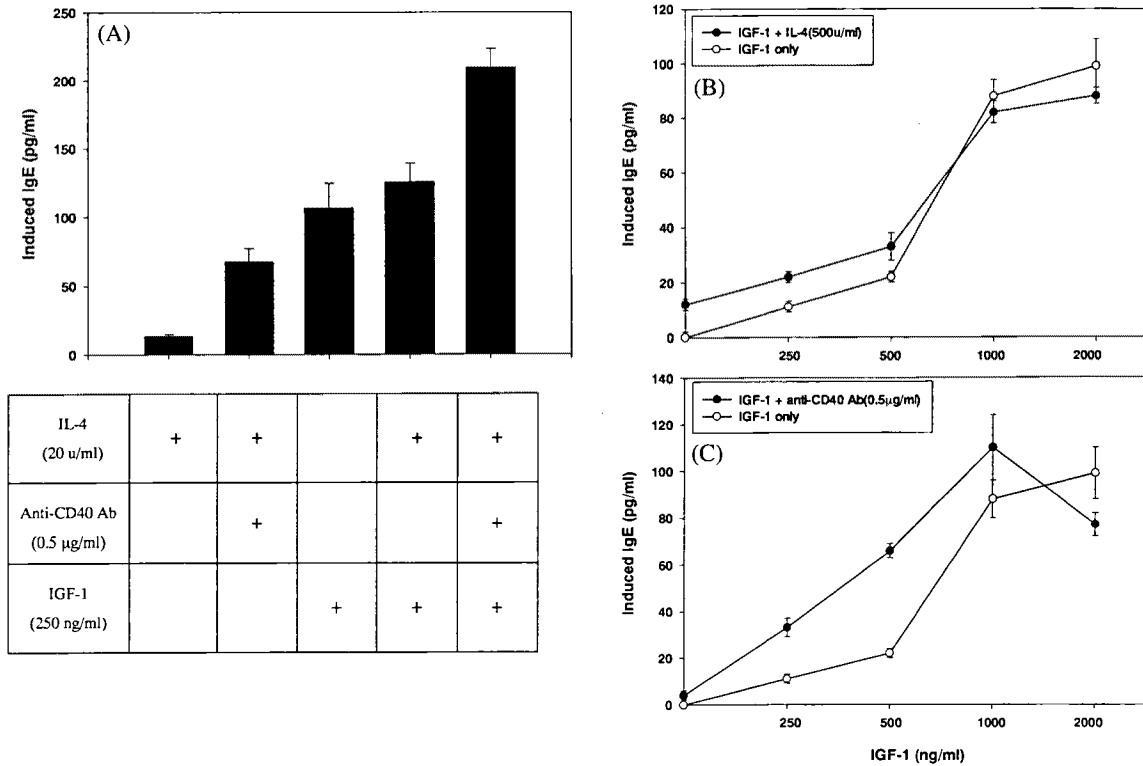
Fig. 2. Panel A. Regulatory effect of IGF-1 on IgE production in human PBMCs. Cells were cultured in the presence of media alone, IL-4 (20 u/ml), anti-CD40 (0.5 μg/ml), IGF-1 (250 ng/ml), or the combinations as indicated. Measurement of induced IgE by ELISA was performed as in Fig 1. Panel B. Effect of IL-4 on the IGF-1-induced IgE production in human PBMCs. Cells were cultured in the presence of media alone or varying concentrations of IGF-1 with or without the addition of IL-4 (500 u/ml). Measurement of induced IgE by ELISA was performed as in Fig 1. Panel C. Effect of anti-CD40 on the IGF-1-mediated IgE production in human PBMCs. Cells were cultured and analyzed for IgE production as in panel B, except that anti-CD40 mAb (0.5 μg/ml) was added instead of IL-4.
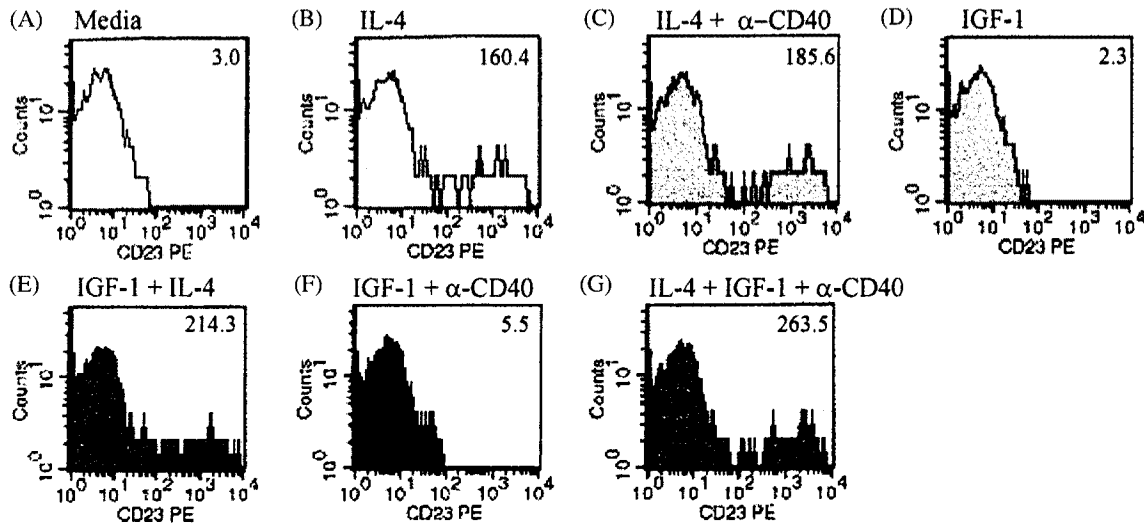

Fig. 3. Enhancing effects of IGF-1 on the IL-4 or IL-4 plus anti-CD40-induced IgERII(Fc R II: CD23) expression. Tonsillar MNCs were cultured in the presence of media alone, IL-4 (100 u/ml), IGF-1 (250 ng/ml), IL-4+IGF-1, with or without anti-CD40 (2 mg/ml) as indicated for 24 h. The surface IgER II (CD23) expression level was determined by FACSCalibur analysis, as described in Materials and Methods. ΔMFI for CD23 was calculated as MFI of CD23 antibody -PE-stained sample minus (-) MFI of the unstained sample. A representative FACS histogram from multiple experiments is shown. Basically the same results were obtained when non-adherent MNCs or purified B cells were used.
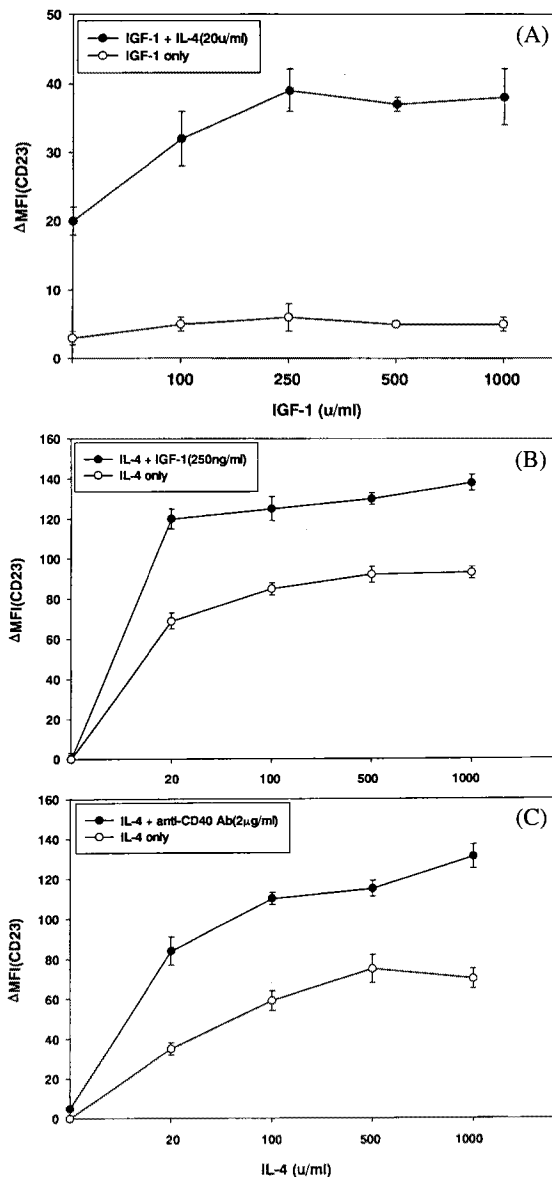
Fig. 5. A schematic diagram of the IgE and IgER II promoters showing transcriptional factors, implicated in IgE/IgER II gene expression and proposed targets for the IL-4 and IGF-1 signal. The (-) numbers represent nucleotide bases upstream from the transcriptional initiation site, which is denoted as +1.

Fig. 4. Panel A. IGF-1 alone does not induce IgER II (CD23) expression, but potentiates the IL-4-induced effect. Tonsillar MNCs were cultured in the presence of media alone or varying concentrations of IGF-1 with or without the addition of IL-4 (20 u/ml) for 24 h. Analysis of the surface CD23 expression was done as in Fig 3. Panel B. Augmentation of the IL-4-mediated IgER II (CD23) expression by IGF-1. Cells were cultured in the presence of media alone or varying concentrations of IL-4 with or without the addition of IGF-1 (250 ng/ml) for 24 h, after which the surface CD23 expression was determined. Panel C. Augmentation of the IL-4-induced IgER II (CD23) expression by anti-CD40 treatment. Cells were cultured and analyzed for CD23 expression as in panel B, except that anti-CD40 mAb (2 mg/ml) was added instead of IGF-1.

2 (TRAF2) (Rothe *et al.*, 1995). A synergistic activation of the IgE promoter via functional interaction between STAT6 and NF-κB was also recently reported (Shen and Stavnezer, 1998).
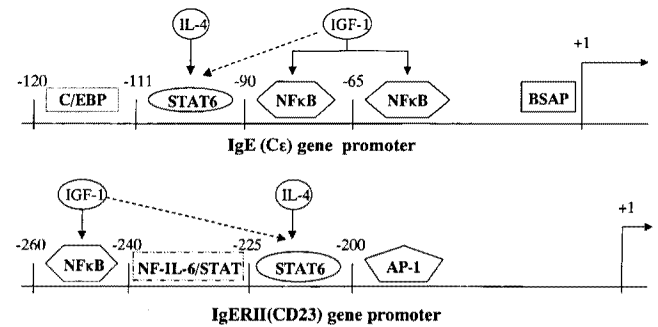
Together, these findings suggest that both STAT6 and NF-κB are major intracellular signaling molecules and transcriptional factors that are primarily responsible for the induction of the IgE and IgER II gene expression. In fact, the comparative examination of IgE (Cε) and IgER II (CD23) promoters reveals that there are common sequence elements which serve as binding sites for STAT6 and NF-κB in close proximity (Fig 5).

Since IGF-1 can modulate the IgE and IgER II expression in a manner similar to IL-4 and CD40 stimulation, we wanted to examine whether or not IGF-1 affects the activation of STAT6 or NF-κB. Using two oligomers that contained the STAT6 binding site (GAS) derived from Cε and CD23 promoter sequences, we performed EMSA. As seen in Fig 6, nuclear extracts from IL-4-stimulated tonsillar lymphocytes contained a nuclear factor which binds to the GAS of both of the promoters (lanes 1 and 2 in Fig 6A and B). The identity of the induced nuclear factor was confirmed to be STAT6. This was concluded by pre-incubating the nuclear extracts with anti-STAT6 antibodies, which resulted in a specific inhibition of the complex formation (lanes 2 and 3). While IGF-1 did not independently induce STAT6 activation, it substantially enhanced the IL-4-induced STAT6 binding to GAS (lanes 4 and 5). Anti-CD40 stimulation alone neither induced STAT6 activation nor positively regulated the IL-4 plus IGF-1 - induced STAT6 activity (lanes 6 and 7). The same pattern of regulation was observed for STAT6 binding to IgE (Cε) and IgER II (CD23) GAS elements (panels A and B). These data suggest that IGF-1 upregulates the IL-4-induced STAT6 binding to the IgE and IgER II promoters to activate transcription. In order to access the role of NF-κB together with STAT6 in IGF-1-mediated IgE and IgER II regulation, we also utilized oligonucleotide probes containing both STAT6 and NF-κB sites representing the longer promoter sequence of IgE and IgER II. As shown in Fig 7, IL-4 specifically induced STAT6 binding, while IGF-1 induced a strong NF-κB binding (lanes 2 and 4). The co-treatment of IL-4 and IGF-1 produced increased STAT6 binding together with

(A) Cε GAS probe　　　**(B) CD23 GAS probe**

1 2 3 4 5 6 7 8　　　1 2 3 4 5 6 7 8
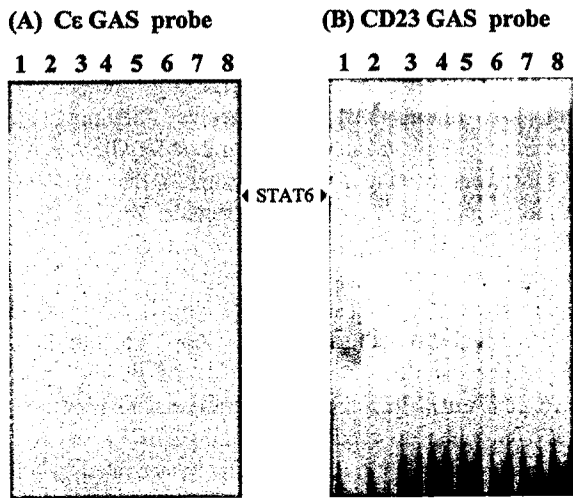
◄ STAT6 ►

IgE(Cε)									IgERII(CD23b)							
-	+	+	-	+	-	+	+	IL-4								
-	-	-	+	+	-	+	+	IGF-1								
-	-	-	-	-	+	+	+	α-CD40	-	-	-	-	-	+	+	+
-	-	-	-	-	-	-	+	Comp.	-	-	-	-	-	-	-	+
-	-	+	-	-	-	-	-	STAT6 Abs	-	-	+	-	-	-	-	-
1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8

Fig. 6. Effect of IGF-1 on STAT 6 binding to IL-4RE(GAS) of IgE and IgER II promoters: IGF-1 enhances the IL-4-induced STAT6 activity. Tonsillar MNCs were stimulated with media alone, IL-4 (100 u/ml), IGF-1 (250 ng/ml), anti-CD40 (2 μg/ml) or combinations thereof for 30 min. Nuclear extracts were prepared and EMSA was performed using IgE(Cε) GAS(panel A) or IgER II (CD23) GAS probe (panel B) that contained a STAT6 binding site. Where indicated, unlabeled competitor oligomers (100×) or polyclonal anti-STAT6 Abs (1 μg) were incubated with the reaction mixture for 30 min prior to the addition of labeled oligomers.

NF-κB binding on the IgER II promoter (lane 5). Thus, it can be noted that IGF-1 independently induced NF-κB activity and potentiated the IL-4-induced STAT6 activity. Basically the same pattern of STAT6 and NF-κB activation was observed with the Cε promoter sequence containing both sites (data not shown).

It appears that while IGF-1 upregulates IL-4-induced IgE and IgER II expression by independently inducing NF-B activation and by augmenting the IL-4-induced STAT6 activation in human primary lymphocytes. Still unidentified additional factors seem to be involved in the independent action of IGF-1 to induce IgE in human PBMCs.

Discussion

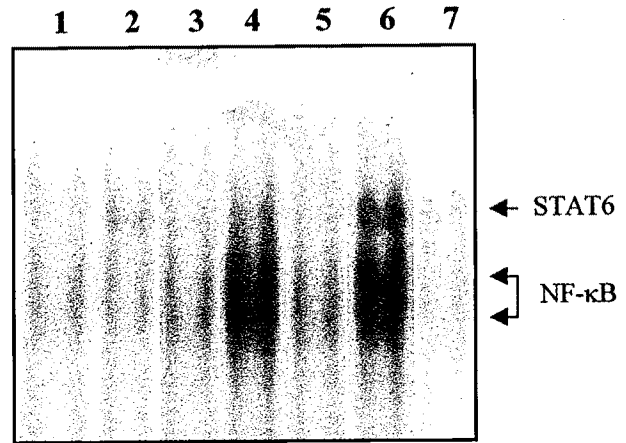1 2 3 4 5 6 7

◄ STAT6

} NF-κB

Fig. 7. Effect of IGF-1 on the activation and binding of STAT6 and NF-κB to the IgER II promoter containing both sites. Tonsillar MNCs were stimulated with media alone (lane 1), 100 u/ml IL-4 (lanes 2 and 3), 250 ng/ml IGF-1 (lanes 4 and 5) or IL-4 plus IGF-1 (lanes 6 and 7) for 30 min, and EMSA was performed as in Fig 6. Anti-STAT6 (lane 3), anti-NF-κB (lane 5) or unlabeled competitor oligomers (lane 7) were included in the reaction mixture in order to confirm the specificity of the complex formation.

While regulatory effects of certain endocrine hormones, growth factors, and neuropeptides on IgE production have been documented sporadically, we report here the novel finding that IGF-1 can modulate both IgE and IgER II expression in human primary lymphocytes. Since the presence of IGF-1 receptors on human PBMCs has already been demonstrated (Kooijman et al., 1992), the effects of IGF-1 on the these cells are apparently mediated by the high-affinity IGF-1 receptor. The primary candidate of the intracellular signaling molecule by IGF-1 receptor signaling is IRS-1/2. It is, upon IGF-1 treatment, shown to be rapidly phosphorylated on multiple tyrosine residues. Docking sites for various downstream signaling molecules with SH2 domains are thus provided. These include: phosphatidyl inositol 3-kinase (PI3K) and Ras/mitogen activated protein kinase (MAPK) module components (Myers et al., 1994).

While the IRS-1/2-mediated signal is thought be involved in IGF-1-mediated growth as well as differentiation response, the enhancing effect of IGF-1 on the IgE and IgERII expression observed in this study is unlikely due to the growth-promoting effect of IGF-1 during the culture period. There was no difference in the cell numbers noted in IGF-1-treated vs. untreated cultures in our experimental conditions. Rather, our data suggest that IGF-1 specifically modulates the IgE and IgER II expression by independently inducing NF-κB and potentiating the IL-4-induced STAT6 activation. This notion has been supported by experiments using inhibitors for NF-κB and STAT6 activation. That is, an anti-oxidant pyrrolidine dithiocarbamate (NF-κB inhibitor) and tyrphostin (Jak tyrosine kinase inhibitor) specifically abrogated the IGF-1-induced NF-κB and STAT6 activity, respectively with a

concomitant inhibition of the IGF-1-induced IgER II (CD23) expression (data not shown). The NF-κB-inducing signal of IGF-1 resembles the CD40-mediated signal, a well-known co-stimulator for IL-4-induced IgE and IgER II expression. Based on the recent finding that MEKK1 can induce NF-κB activity via inhibitor κB (I κB) kinase activation (Baumann et al., 2000), the observed NF-κB activation by IGF-1 in this study may occur through IRS-1/2. The Ras/MEKK/MEK/MAPK pathway has been strongly suggested as a major down-stream target of IRS-1/2 (Myers et al., 1994).

Another important novel finding in this study is the augmentation of the IL-4-induced STAT6 activation by IGF-1 as clearly demonstrated in Fig 6 and Fig 7. Although the exact mechanism of the IGF-1-mediated potentiation of STAT6 remains elusive, the following possibilities are raised: First, co-treatment of IL-4 and IGF-1 can facilitate transactivation of the protein tyrosine kinase Jak1 by auto(trans) phosphorylation, a major Jak family member known to activate STAT6. In this regard, it has been reported that Jak1 and Jak3 associate with the IL-4 receptor α and chain, respectively (Nelms et al., 1999). It has been recently demonstrated that Jak1 and Jak2 both interact with the IGF-1 receptor (Gual et al., 1998). Second, although it has not been clearly demonstrated, the activation of STATs by the receptor tyrosine kinase itself is a very plausible scenario. In fact, a growing body of data indicates that many hormones or cytokines utilizing the receptor tyrosine kinase can induce STAT activation (Leonard and O'Shea, 1998). Thus, the direct activation of STAT6 by a IGF-1 receptor kinase by tyrosine phosphorylation can be an alternative mechanism to enhance the IL-4R/Jak-1-mediated STAT6 activation. Finally, the augmentation of STAT6 activation by serine/threonine phosphorylation can be induced by the downstream kinases of IRS-1/2. The potential candidates include PI3K, ribosomal S6 kinase (RSK), or MAPK (Wang et al., 1993; Rondinone et al., 1997). The requirement of serine phosphorylation for the maximal activation of STATs is well established (Wen et al., 1995). In particular, the phosphorylation of Ser727 of STAT1 has been strongly implicated for the potentiation of transcriptional activity by promoting the interaction of STAT1 with other transcriptional factors (Zhang et al., 1998). For STAT6, phosphorylation of multiple serine/threonine residues has also been reported, although the biological significance of this phenomenon is unclear (Pesu et al., 2000).

Our observation that IGF-1 alone can induce IgE production, but not IgER II expression, raises several speculations regarding the differential molecular mechanism of IgE and IgER II regulation. While STAT6 and NF-κB are common major transcriptional factors that are strongly implicated in both the IgE and IgER II gene regulation, the relative contribution of these two factors to the transcriptional activation of IgE and IgER II genes may be different. This is suggested in a somewhat different organization of binding sites for these two factors in the IgE and IgER II promoters, in that there are tandem NF-κB sites next to each other in the IgE

promoter. However, there is only one NF-κB site on the IgER II promoter. Also while only a single perfect STAT6 binding site is found on both the IgE and IgER II promoters, a close examination reveals that there are two additional GAS (imperfect STAT6 site) next to the major STAT6 site on the IgER II promoter (Fig 5). These suggest that the NF-κB-activation signal acts as a stronger inducer for IgE than for IgER II. Whereas, the STAT6-activation signal acts as a stronger inducer for IgER II than for IgE. This probably explains in part why IGF-1 (a strong NF-κB activator) alone can induce IgE, but not IgER II. Also, IGF-1 co-stimulates the IgER expression only in the presence of IL-4 (a primary STAT6 activator). Still, other transcriptional factors, such as the CAAT-enhancer binding protein (C/EBP) or B cell-specific activator protein (BSAP), may be activated by a IGF-1-mediated signal. It can also contribute to the IGF-1-induced IgE production (Fig 5, Stein et al., 1993; Thienes et al., 1997). The role of the unique IGF-1 signal that is independent of IRS-1/2 also needs to be considered. The IRS-independent activation of Ras/MAPK is suggested in the IGF-1-induced differentiation response via action of the IGF-1 receptor-bound Shc, which in turn leads to the activation of the Ras/MAPK pathway (Dey et al., 1996; Valentinis et al., 1999). The fact that insulin induced no detectable level of IgE, nor potentiated the IgER II in our system (data not shown), supports the possibility that the observed IGF-1 action on IgE and IgER II regulation may occur through IRS-1/2-independent pathways. Another possibility resides in the different culture systems and culture durations employed to assay for IgE and IgER II. For IgE production, a prolonged incubation (14 days) of PBMCs was necessary. A relatively short culture time (1 day) was sufficient to assay IgER II induction by tonsillar MNCs or PBMCs. During the prolonged incubation of PBMCs, a small amount of IL-4, which might have been produced by T cells in the culture, would have contributed to the IgE production in IGF-1-treated samples.

In conclusion, the present study demonstrates the novel function of IGF-1 in modulating the IgE and IgER II expression via activation of NF-κB and STAT6. A detailed investigation of the molecular mechanisms of IGF-1 in modulating the IgE and IgER II expression would add valuable information to our limited knowledge of the regulation of the complex in vivo IgE response.

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