**Brief Definitive Report**

**Growth Hormone and Insulin-like Growth Factor I Induce Immunoglobulin (Ig)E and IgG4 Production by Human B Cells**

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**Summary**

We studied the effects of growth hormone (GH), insulin-like growth factor I (IGF-I), IGF-II, and insulin on human immunoglobulin E (IgE) and IgG4 production. GH and IGF-I induced IgE and IgG4 production by normal donors' mononuclear cells (MNC) depleted of slgE⁺ and slgG4⁺ B cells without affecting IgM, IgG1, IgG2, IgG3, IgA1, or IgA2 production, whereas IGF-II and insulin failed to do so. GH-induced IgE and IgG4 production was specific, and was not mediated by IGF-I, interleukin 4 (IL-4), or IL-13, since it was blocked by anti-GH antibody (Ab), but not by anti-IGF-I Ab, anti-IL-4 Ab, or anti-IL-13 Ab. Conversely, IGF-I-induced IgE and IgG4 production was blocked by anti-IGF-I Ab, but not by anti-GH Ab, anti-IL-4 Ab, or anti-IL-13 Ab. Moreover, interferon α (IFN-α) or IFN-γ, which counteracted IL-4- and IL-13-induced IgE and IgG4 production, had no effect on induction by GH or IGF-I. In contrast to MNC, GH or IGF-I failed to induce IgE and IgG4 production by purified slgE⁻, slgG4⁻ B cells. However, in the presence of anti-CD40 monoclonal antibody (mAb), GH or IGF-I induced IgE and IgG4 production by these cells. Purified slgE⁺, but not slgE⁻, B cells from atopic patients spontaneously produced IgE. GH or IGF-I with anti-CD40 mAb induced IgE production by slgE⁺ B cells, whereas they induced IgE production by slgE⁻ B cells. Similarly, whereas GH or IGF-I with anti-CD40 mAb failed to enhance IgG4 production by slgG4⁺ B cells from atopic patients, they induced IgG4 production by slgG4⁻ B cells. Again, neither IgE nor IgG4 induction was blocked by anti-IL-4 Ab or anti-IL-13 Ab. These results indicate that GH and IGF-I induce IgE and IgG4 production by class switching in an IL-4- and IL-13-independent mechanism.

In mononuclear cells (MNC), IL-4 induces IgE and IgG4 production, which can be inhibited by IFN-α or IFN-γ (1–3). In contrast, IL-4 alone cannot induce IgE and IgG4 production by purified B cells. However, IL-4 plus anti-CD40 mAb induces IgE and IgG4 production which is not inhibited by IFN-α or IFN-γ (3–6). We and others (7, 8) have reported that T cells from patients with hyper IgE syndrome or atopy secrete IgE-enhancing activity that was not IL-4. We also found that IgE and IgG4 production was modulated by erythropoietin and neuropeptides in an IL-4-, IFN-α-, and IFN-γ-independent fashion (9, 10). These results indicate that there may be another IgE- and IgG4-inducing cytokine(s). Indeed, IL-13 has been shown to induce IgE and IgG4 production in IL-4-independent mechanisms although there are commonalities between the IL-4 and IL-13 receptor, and it is still possible that other factors may be involved in induction of IgE and IgG4 production (11, 12).

We have recently reported that growth hormone (GH) and insulin-like growth factor I (IGF-I) enhanced IgE and IgG4 production by human plasma cell line and plasma cells, whereas IGF-II or insulin failed to do so (13). We therefore studied whether GH and IGF-I would induce IgE and IgG4 production by normal B cells. Because hormones in serum modulated the GH-induced response, we compared the effects of peptides in serum- and hormone-free medium, DME/F-12 (13, 14). We show that GH and IGF-I, but not IGF-II or insulin, induce IgE and IgG4 production by tonsillar slgE⁺, slgG4⁻ B cells in an IL-4- and IL-13-independent fashion.

**Materials and Methods**

**Reagents.** The following recombinant human cytokines and Abs were kindly provided by companies described previously (2, 3): IL-4 and rabbit anti-IL-4 Ab (Ono Pharmaceutical Company, Osaka, Japan), IFN-α and IFN-γ (Takeda Chemical Industries, Osaka, Japan), and GH and rabbit anti-GH Ab (Sumitomo Pharmaceuticals, Osaka, Japan). Mouse IgG1 anti-GH receptor mAb (MAB 263) was purchased from Agen Biomedical Ltd. (Qld, Australia) (13). Human recombinant IL-13 was purchased from Pepro Tech Inc. (Rocky Hill, NJ). Human recombinant IGF-I, IGF-II, insulin, rabbit anti-IGF-I Ab, mouse IgG1 anti-IGF-I receptor mAb (aIR-3), mouse IgM anti-CD40 mAb (MA6), rabbit anti-IL-13 Ab, control rabbit IgG, and control mouse IgG1 were purchased from...
Results

Preliminary experiments have shown that in MNC, medium alone failed to induce IgE (<0.15 ng/ml, n = 10) or IgG4 (<0.3 ng/ml, n = 10) production, whereas GH and IGF-I at 250 ng/ml induced IgE (4.1 ± 2.7 and 5.2 ± 2.4 ng/ml, respectively, n = 10) and IgG4 (33.0 ± 7.4 and 41.7 ± 7.2 ng/ml, respectively, n = 10) production. To rule out the possibility that GH- and IGF-I-induced IgE and IgG4 production may result from the expansion of a small slgE+ and slgG4+ B cell population, MNC were depleted of slgE+ and slgG4+ B cells, and they were cultured with peptides. As shown in Fig. 1, A–H, GH and IGF-I induced IgE and IgG4 production in a dose-dependent fashion, whereas they failed to induce IgM, IgG1, IgG2, IgG3, IgA1, and IgA2 production. In contrast, IGF-II and insulin failed to induce IgE (<0.15 ng/ml) or IgG4 (<0.3 ng/ml) or other Ig production (Fig. 1, A–H).

It has been reported that GH-induced stimulation was mediated by endogenously produced IGF-I (17, 18), although we and others (13, 19, 20) have reported the direct enhancing effect of GH. It has also been reported that IL-4 and IL-13 induced IgE and IgG4 production, and that IFN-α and IFN-γ antagonized induction by them (1, 3, 11, 12, 21). Therefore, we compared the inducing effects of GH and IGF-I with those of IL-4 and IL-13. As shown in Fig. 2, GH-induced IgE and IgG4 production was blocked by anti-GH Ab, whereas neither anti-IGF-I Ab nor anti-IL-4 Ab nor anti-IL-13 Ab did so. Addition of anti-GH receptor Ab also blocked induction, whereas anti-IGF-I receptor mAb failed to do so (data not shown). Moreover, neither IFN-α nor IFN-γ antagonized the effect of GH (Fig. 2). Conversely, IGF-I–induced IgE and IgG4 production was blocked by anti-IGF-I Ab, whereas none of the anti-GH Ab, anti-IL-4 Ab, anti-IL-13 Ab, IFN-α, or IFN-γ had any effect. Addition of anti-IGF-I receptor mAb also blocked induction whereas anti-GH receptor mAb failed to do so (data not shown). In contrast, IL-4– and IL-13–induced IgE and IgG4 production was blocked by anti-IL-4 Ab and anti-IL-13 Ab, respectively, but not by anti-GH or anti-IGF-I Ab, and induction was antagonized by IFN-α and IFN-γ (Fig. 2).

We also measured GH, IGF-I, and IL-4 concentrations in culture supernatants. To do this, MNC were cultured with or without GH (250 ng/ml) for measurement of IGF-I or IL-4, and, conversely, they were cultured with or without IGF-I (250 ng/ml) for measurement of GH or IL-4. On the other hand, they were cultured with or without IL-4 (1,000 U/ml) for measurement of GH or IGF-I. Cultured supernatants did not contain detectable levels of GH (<10 pg/ml),
IGF-I (<30 pg/ml), or IL-4 (<40 pg/ml), and addition of GH, IGF-I, or IL-4 failed to induce them.

We next studied the effects of GH and IGF-I on IgE and IgG4 production by purified B cells with or without anti-CD40 mAb (2, 4-6). As shown in Fig. 3, slgE- , slgG4- B cells failed to produce detectable levels of IgE or IgG4. Neither anti-CD40 mAb nor GH induced them. However, GH plus anti-CD40 mAb induced IgE and IgG4 production which was blocked by anti-GH Ab and anti-GH receptor mAb (data not shown), whereas none of the anti-IGF-I Ab, anti-IGF-I receptor mAb (data not shown), anti-IL-4 Ab, or anti-IL-13 Ab did so. Similarly, IGF-I plus anti-CD40 mAb induced IgE and IgG4 production, whereas IGF-I alone failed to do so. Induction by IGF-I plus anti-CD40 mAb was specifically blocked by anti-IGF-I Ab or anti-IGF-I receptor mAb (data not shown), but not by anti-GH Ab, anti-GH receptor mAb (data not shown), anti-IL-4 Ab, or anti-IL-13 Ab. Moreover, no detectable levels of GH, IGF-I or IL-4 were induced in cultures of these B cells. In contrast to GH and IGF-I, neither IGF-II plus anti-CD40 mAb nor insulin plus anti-CD40 mAb induced IgE (<0.15 ng/ml) or IgG4 (<0.3 ng/ml) production by slgE- , slgG4- B cells.

We then studied the effects of GH and IGF-I on spontaneous IgE and IgG4 production by atopic patients' B cells.

**Figure 2.** Specificity of the effects of GH and IGF-I on IgE and IgG4 production by MNC from nonatopic donors. MNC from nonatopic donors were depleted of slgE+ and slgG4+ B cells, and were cultured (3 x 10^6/well) with indicated factors. GH was used at 250 ng/ml, IGF-I at 250 ng/ml, IL-13 at 1,000 U/ml, IL-13 at 50 ng/ml, and all the Abs at 10 µg/ml, IFN-α at 1,000 U/ml and IFN-γ at 1,000 U/ml. After 14 d of culture, IgE (A) and IgG4 (B) production were determined. Values are means ± 1 SD of triplicate cultures.

**Figure 3.** Effects of GH and IGF-I on IgE and IgG4 production by B cells from nonatopic donors. B cell from nonatopic donors were depleted of slgE+ and slgG4+ cells (slgE- , slgG4- B cells), and they were cultured (10^6/well) with indicated factors. Anti-CD40 mAb were used at 0.1 µg/ml, GH at 250 ng/ml, IGF-I at 250 ng/ml, and all the Abs at 10 µg/ml. After 14 d of culture, IgE ( ) and IgG4 ( ) production were determined. Values are means ± 1 SD of triplicate cultures.
To do this, sIgE⁺, sIgE⁻, sIgG4⁺, and sIgG4⁻ B cells from atopic patients were separated and cultured with medium or factors. As shown in Fig. 4 A, sIgE⁺ B cells spontaneously produced IgE, which was not enhanced by GH plus anti-CD40 mAb or by IGF-I plus anti-CD40 mAb. (Expts. 1–3). Fig. 4 A also showed that none of anti-GH or anti-IGF-I Ab (Expt. 1), or anti-IL-4 or anti-IL-13 Ab (Expt. 2), or IFN-α or IFN-γ (Expt. 3) affected IgE production by sIgE⁺ B cells. In contrast, sIgE⁻ B cells did not produce IgE with medium alone. However, GH plus anti-CD40 mAb or IGF-I plus anti-CD40 mAb induced IgE production by sIgE⁻ B cells, which was blocked by anti-GH and anti-IGF-I Ab, respectively (Expt. 1), but not by anti-IL-4 or anti-IL-13 Ab (Expt. 2), or by IFN-α or IFN-γ (Expt. 3).

Identical results were observed in IgG4 production by sIgG4⁺ or sIgG4⁻ B cells. As shown in Fig. 4 B, neither GH plus anti-CD40 mAb nor IGF-I plus anti-CD40 mAb affected IgG4 production by sIgG4⁺ B cells, whereas they induced IgG4 production by sIgG4⁻ B cells. Induction by GH and IGF-I was specifically blocked by anti-GH Ab and anti-IGF-I Ab, respectively (Expt. 1), whereas it was not blocked by any of the anti-IL-4 Ab or anti-IL-13 Ab (Expt. 2), or by IFN-α or IFN-γ (Expt. 3).

Discussion

We have demonstrated that GH and IGF-I specifically induced IgE and IgG4 production by normal donors’ MNC depleted of sIgE⁺ and sIgG4⁺ B cells, whereas IGF-II and insulin failed to do so. The detailed mechanisms of differential effects of these peptides are currently under investigation. However, this is not surprising. We and others (13, 22, 23) have reported that GH and IGF-I stimulated plasma cells, pre-T cells, and neutrophils, whereas IGF-II or insulin were either less stimulatory or without effect.

It has been reported that GH-induced stimulation was mediated by IGF-I, although a direct stimulating effect of GH was also reported (13, 17–20). Moreover, IL-4 and IL-13 have been shown to induce IgE and IgG4 production (1–3, 11, 12). However, our results indicate that GH effect was specific, and was not mediated by IGF-I, IL-4, or IL-13, because GH-induced IgE and IgG4 production was blocked by anti-GH Ab, but not by anti-IGF-I Ab, anti-IL-4 Ab, or anti-IL-13 Ab. In addition, whereas IFN-α and IFN-γ inhibited IL-4- and IL-13-induced IgE and IgG4 production, they had no effect on GH- or IGF-I-induced IgE and IgG4 production. Furthermore, no IGF-I or IL-4 was detectable in the culture supernatants with GH. Conversely, IGF-I-induced induction was not mediated by GH, IL-4, or IL-13, because it was blocked by anti-IGF-I Ab, but not by anti-GH Ab, anti-IL-4 Ab or anti-IL-13 Ab. Moreover, no GH or IL-4 was produced by IGF-I.

GH and IGF-I also induced IgE and IgG4 production by normal donors’ sIgE⁻, sIgG4⁻ B cells in the presence, but not absence, of anti-CD40 mAb. Again, this induction was specific to each peptide. These results indicate that GH- and IGF-I-induced IgE and IgG4 production was due to switching but not to expansion of sIgE⁺ and sIgG4⁺ B cells. This was directly shown by using sIgE⁺, sIgG4⁺, sIgE⁻, or sIgG4⁻ B cells from atopic patients. Whereas GH plus anti-CD40 mAb or IGF-I plus anti-CD40 mAb induced IgE and IgG4 production by sIgE⁻ and sIgG4⁻ B cells, respectively, they...
failed to do so by SlG4- and SlG4+ B cells, respectively. The in vivo influence of GH and IGF-I remains to be elucidated. However, it has been reported that alveolar macrophages from patients with lung disease produced IGF-I, and that IGF concentrations in bronchoalveolar lavage (BAL) fluid was elevated in those patients (24, 25). In accordance with this, we have found that GH and IGF-I concentrations in BAL fluid were elevated in asthmatic patients: GH and IGF-I concentrations in BAL fluid in asthmatic patients (n = 5) were 45 ± 12 and 61 ± 10 pg/ml (n = 5), respectively, whereas those in normal donors (n = 5) were <10 and <30 pg/ml, respectively. Moreover, there are cases with IGF deficiency and GH deficiency and low serum levels of IGF-I (26).

GH and IGF-I seem to be excellent reagents in the study of IgE and IgG4 regulation. We and others (1-3, 5, 21) have reported that various factors modulate IL-4-induced IgE and IgG4 production. The interaction of GH and IGF-I with those factors is currently under investigation.

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