Growth Hormone Receptors and Insulin-like Growth Factor I Receptors on Human Peripheral Blood Cells

Noboru Igarashi and Satoshi Takakuwa
Department of Pediatrics, Kanazawa University School of Medicine, Ishikawa, Japan

Abstract Growth hormone (GH) and insulin-like growth factor I (IGF-I) may be able to modulate some functions of the immune system. In this study, we examined the expression of the receptors for GH and IGF-I on human peripheral blood cells in physiological and pathological conditions.

By using two-colour analysis on flow cytometry with a biotin-avidin system, there was a specific GH binding site on human peripheral lymphocytes. GH-binding was more prominent on CD20+ (B cells) than on CD2+ (T cells), although GH receptor messenger ribonucleic acid (mRNA) could be detected in the T cells as well as in the B cells. There was no age-dependency of GH-binding on the CD20 cells, and there was no relationship between GH-binding and serum GH-binding protein activities, which reflect the tissue levels of GH receptors.

We detected aIR3, anti IGF-I receptor antibody, bindings and IGF-I receptor mRNA expression in peripheral blood cells. Based on two-colour analysis, there were relatively higher bindings of aIR3 on CD4+ and CD16+ cells, intermediate bindings on CD8+ cells and weak bindings on CD20+ cells. In cord blood cells, aIR3 bindings and IGF-I receptor mRNA were higher than in adult cells. In GH-deficient patients, aIR3 bindings were higher than in control subjects, and reverted to normal after GH therapy.

These results suggest that 1) GH and IGF-I may have hormonal effects on the immune system, 2) GH receptors on lymphocytes do not correlate with the tissue levels of GH receptors, and 3) the cellular levels of IGF-I receptors may be inversely related to the ambient IGF-I concentrations.

Key words: growth hormone receptor, insulin-like growth factor I receptor, immune system

Introduction

Recent studies have suggested that growth hormone (GH) and insulin-like growth factor I (IGF-I) constitute part of the neuroendocrine immune system. GH and IGF-I stimulate cellular proliferation of lymphoid organs as well as activation of peripheral lymphocytes and macrophages (1). There are specific receptors for GH and IGF-I, and local production of GH and IGF-I by peripheral lymphocytes and macrophages has been reported (1). Thus, the cells of the immune system may be important...
targets of the GH-IGF-I axis. In this study, we investigated the receptor dynamics for GH and IGF-I on human peripheral blood cells in physiological and pathological conditions.

Material and Methods

Flow Cytometric Analysis of GH Receptor (GHR)

Peripheral lymphocytes (PBL) were isolated by the Ficoll-Hypaque method, and incubated with Biotin-labeled GH and fluorescein isothiocyanate (FITC)-labeled cell surface marker (CD2/CD20). After washing, the cells were stained with PE-conjugated streptavidin, followed by two-color analysis on flow cytometry.

Flow Cytometric Analysis of IGF-I Receptor (IGF-IR)

Whole blood was incubated with αIR3, a monoclonal antibody against type I IGF receptor (Oncogene Science Co.). After washing, the cells were treated with biotinylated goat antimouse Ig. The cells were then stained with streptavidin phycoerythrin (SAPE) and/or FITC-conjugated specific cell marker (CD4/CD8/CD16/CD20) and analyzed by flow cytometry.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) of GHR and IGF-IR mRNA

Total RNA was extracted from peripheral blood cells, (lymphocytes, monocytes, and granulocytes) by single-step methods using the ribonucleic acid-zol (RNAzol) technique. After reverse transcription of $4 \times 10^5$ blood cells RNA using oligo-dt, polymerase chain reaction (PCR) was performed by using GHR or IGF-IR specific primer pairs. After electrophoresis on agarose gel, amplified complementary deoxyribonucleic acids (cDNAs) were blotted onto nylon membranes by vacuum transfer and hybridized to digoxigenin (DIG)-labeled GHR or IGF-IR cDNA probes [kindly provided by American type culture collection (ATCC)]. RT-PCR products of β-actin were use as the internal standard.

Serum GH-binding Protein (GHBP) Activities

GHBP was determined by the immunoprecipitation method as described previously (2).

Western Blotting of IGF-IR Protein on Blood Cell Surface

Streptavidin blotting was performed as reported by Levy-Toledano (3). In brief, the cell surface IGF-IR was biotinylated with N-hydroxy succinimide long chain (NHS-LC)-biotin, and then was immunoprecipitated with αIR3. Immune complexes were precipitated with protein-A agarose and were prepared for Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). After electroblocting, the receptor proteins on the membrane were detected by Enhanced Chemiluminescent (ECL) methods (Amersham Co.).

Subjects

Peripheral blood cells were obtained from the cord blood of full-term neonates and from samples taken from healthy adults and patients with GH deficiency, insulin-dependent diabetes mellitus (IDDM), Turner syndrome,
and chronic renal failure (CRF).

**Results**

When the whole PBL were stained with biotinylated-GH, there were biotin-GH binding positive cells and negative cells (Fig. 1). Two-color analysis by flow cytometry suggested that biotin-GH binding was more prominent on CD20+ cells (=B cells) than CD2+ cells (=T cells) (Fig. 2). Biotin-GH binding on CD20+

![Figure 1](image1.png)

**Fig. 1** Biotin-GH binding to whole peripheral lymphocytes

![Figure 2](image2.png)

**Fig. 2** Biotin-GH binding to lymphocyte subpopulation

![Figure 3](image3.png)

**Fig. 3** Displacement of biotin-GH binding on CD20+ cells in the presence of unlabeled-GH

cells was specifically displaced by non-labeled GH in a dosedependent manner (Fig. 3). By using RT-PCR methods, we could detect GHR mRNA in T cells as well as in B cells, and there was abundant GHR mRNA in Ebstein

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Barr Virus (EBV) transformed B cells and in IM-9 cells (=B cell line) (Fig. 4). There was no age-dependency for biotin-GH binding on CD20+ cells (Fig. 5). mRNA expressions in lymphocytes and granulocytes from cord blood were equal to those from adult controls (Fig. 6). Biotin-GH binding on CD20+ cells from patients with GH-deficiency, Turner syndrome, IDDM and CRF were similar to those of control subjects. (Fig. 7). Previously, we reported the case of a GH-resistant dwarf with severely reduced activity of serum GHBP (Fig. 8). In this case, biotin-GH binding on CD20+ cells was equal to that of controls (Fig. 9), and GHR mRNA levels in blood cells were similar to those of control subjects (Fig. 10). There was no relationship between serum GHBP activities and biotin-GH binding levels on CD20+ cells (Fig. 11).

There were αIR3 bindings on lymphocytes, monocytes, granulocytes, and red blood cells (Fig. 12). IGF-IR proteins were also detected on the surface of lymphocytes and granulocytes as well as on EBV-transformed B cells by western blotting (Fig. 13). IGF-IR
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**Fig. 8** GH-resistant short with severely reduced GH-binding protein

**Fig. 9** Biotin-GH binding on CD20+ cells of the GH-resistant case
mRNA was detected in these peripheral blood cells by RT-PCR methods (Fig. 14). Based on two-color analysis of lymphocyte subpopulations, higher bindings of αIR3 were detected on CD4+ helper cells and CD16+ natural killer cells, intermediate bindings on CD8+ suppressor cells and lower bindings on CD20+ B cells (Fig. 15). IGF-IR expressions on lymphocytes, monocytes and neutrophils were more prominent on cord blood cells than on adult cells (Fig. 16). Two-color analysis indicated that αIR3 bindings on CD16 and CD20 cells from cord blood were higher than on adult cells (Fig. 12).
Fig. 14  IGF-I receptor expressions in blood cells

Fig. 15  αIR3 bindings on lymphocyte subpopulations

Fig. 16  αIR3 bindings on lymphocyte subpopulations of cord and adult

Fig. 17  αIR3 bindings on lymphocyte subpopulations of cord and adult

Fig. 18  IGF-I receptor mRNA expressions in the blood cells of cord and adult
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Fig. 19 αIR3 bindings on lymphocyte subpopulations of the GH deficient cases and control

Fig. 20 αIR3 bindings on the blood cells before and after GH therapy in GH deficient case

17). IGF-IR mRNA expressions in cord blood cells were also higher than in adult cells (Fig. 18). In GH deficient patients, αIR3 bindings on each lymphocyte subpopulations were higher than on control cells (Fig. 19). In GH deficient patients, αIR3 bindings on blood cells decreased after GH replacement therapy (Fig. 20).

Discussion

By using flow cytometric analysis, we detected specific biotin-GH bindings as GH receptors on human peripheral lymphocytes, as previously described by Kiess (4). Although GHR mRNA was detected in T cells as well as in B cells, GHR expressions on B cells were more prominent than on T cells. Previous reports suggest that GH may stimulate not only humoral immunity but also cellular immunity (1, 5). Our results conflicted with these data. It remains to be elucidated whether the regulatory role of GH in the immune system is mediated through its specific receptor or not.

There was no age-dependency of biotin-GH bindings on CD20+ lymphocytes, and there was no relationship between biotin-GH bindings on lymphocytes and serum GHBP activities. It has been suggested that GHBP is derived from extracellular components of tissue GH receptors, and its serum activities were correlated with tissue levels of GH receptor (6). These results indicate that GHR on lymphocytes do not reflect the GH receptor dynamics in peripheral tissues. In contrast, Clot reported that a GH-dependent increment of pyruvate dehydrogenase activity in PBL was not observed in patients with Laron-type dwarfism, who have extreme resistance to GH due to GH receptor defect (7). Merimee reported that GH-stimulated IGF-I secretion by B lymphocytes from Pygmies, who are GH-resistant, was lower than in controls (8). These results suggest that the cellular response to GH can be used to investigate GH-resistance. The clinical significance of GHR on PBL requires further study.

There were IGF-IR on peripheral blood cells studied by flow cytometric analysis as described by Kooijman (9). Furthermore, we detected IGF-IR protein on the blood cell surface using Western blotting and IGF-IR mRNA
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in these cells by RT-PCR. The role of IGF-I in the immune system has been shown to involve 1) stimulation of lymphocyte proliferation, 2) activation of T cells and B cells function, and 3) priming of neutrophils for super oxide anion secretion and other functions (1). These actions of IGF-I are thought to be mediated via specific receptors on blood cells.

IGF-IR on cord blood cells were higher than on adult blood cells, as reported by Rosenfeld in studies using radioligands (10). Since the increase in IGF-IR expression of cord cells was associated with an increase in IGF-IR mRNA levels, the increased IGF-IR is most likely due primarily to increased receptor biosynthesis. The presence of increased IGF-IR in cord blood cells suggests a mechanism by which maximal fetal growth can occur at levels of IGF-I that are lower than postnatal levels. Further studies are necessary to examine quantitative and qualitative differences in cellular responses to IGF-I between neonatal and adult cells.

In GH deficient patients, IGF-IR expressions on PBL were more prominent than on control cells, and decreased after GH therapy. Eshet reported the similar dynamics of IGF-IR on RBC and IGF-IR mRNA levels in the lymphocytes of GH deficient and GH resistant cases (11, 12). These results indicate that the in vivo regulation of cellular IGF-IR is by IGF-I itself, as the level of cell surface IGF-IR appears to be inversely related to the ambient IGF-I concentration. IGF-IR expressions on peripheral blood cells may be a useful clinical marker for evaluating the function of the GH-IGF-I axis.

References

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