B Cells from Patients with Graves’ Disease Aberrantly Express the IGF-1 Receptor: Implications for Disease Pathogenesis

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Graves’ disease (GD) is an autoimmune process involving the thyroid and connective tissues in the orbit and pretibial skin. Activating anti-thyrotropin receptor Abs are responsible for hyperthyroidism in GD. However, neither these autoAbs nor the receptor they are directed against have been convincingly implicated in the connective tissue manifestations. Insulin-like growth factor-1 receptor (IGF-1R)-bearing fibroblasts overpopulate connective tissues in GD and when ligated with IgGs from these patients, express the T cell chemokine-expressing, IL-16, and RANTES. Disproportionately large fractions of peripheral blood T cells also express IGF-1R in patients with GD and may account, at least in part, for expansion of IGF-1R⁺ memory T cells. We now report a similarly skewed B cell population exhibiting the IGF-1R⁺ phenotype from the blood, orbit, and bone marrow of patients with GD. This expression profile exhibits durability in culture and is maintained or increased with CpG activation. Moreover, IGF-1R⁺ B cells produce pathogenic Abs against the thyrotropin receptor. In lymphocytes from patients with GD, IGF-1 enhanced IgG production (p < 0.05) and increased B cell expansion (p < 0.02) in vitro while those from control donors failed to respond. These findings suggest a potentially important role for IGF-1R display by B lymphocytes in patients with GD in supporting their expansion and abnormal Ig production. The Journal of Immunology, 2008, 181: 5768–5774.

B lymphocytes play a critical role in normal immunity and the development of autoimmune disease (1–3). They initiate and support immune function through Ag presentation, expression of costimulatory molecules, and production of Abs (1, 4–6). Moreover, B cells can develop into Ig-producing plasma cells, which not only produce host defense, but can also recognize host tissue, leading to autoimmunity (2). AutoAb production plays a central role in the pathogenesis of several of these diseases, such as rheumatoid arthritis and Graves’ disease (GD)⁴ (2, 7). In addition to Ig production and Ag presentation, B cells secrete many cytokines, and thus they modulate immune activation. Among these cytokines are IL-6, TNF-α, and IL-10, all of which can condition T cell responses (5, 8, 9). B cell Ag presentation is robustly up-regulated through the CD40/CD40L (CD154) pathway, which is also essential to T cell activation (10). The concept that B cells play important roles in autoimmune diseases is consonant with the effective interruption of disease progression and activity following therapeutic B cell depletion (11, 12).

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3 Abbreviations used in this paper: GD, Graves’ disease; IGF-1R, insulin-like growth factor-1 receptor; MFI, mean fluorescence intensity; TAO, thyroid-associated ophthalmopathy; TSHR, thyrotropin receptor.

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hematopoietic cell growth and differentiation and normal immune function (19). Peripheral blood T and B cells and monocytes from control human donors express low levels of IGF-1R in vivo (25, 26). Administration of IGF-1 increases the circulating pool of CD4+ T cells and splenic B cells in mice (27, 28), suggesting a role for this growth factor in hematopoietic cell expansion (29). It promotes T cell proliferation during early activation (30) and inhibits apoptosis of both immature and mature T cells through at least three distinct mechanisms (31, 32). With regard to B cell expansion, IGF-1 plays an essential role in the development of bone marrow CD34+ cells into pro-B cells (33). Additionally, IGF-1 increases expression of the IL-4-induced type II IGF receptor (FceRII/CD23) by both human primary immune cells and established B cell lines. This suggests a role in B cell activation and function (34).

We have recently reported that T lymphocytes from patients with GD represent a cell population skewed toward the CD3+ IGF-1R+ phenotype (35). This disproportionate fraction of IGF-1R-displaying T cells can be traced to discrete subsets. In particular, the increased receptor expressing population results from an expanded memory CD45RO+ T cell population. This skew was found in peripheral blood and also in cells harvested from disease-involved orbital connective tissue (35). In contrast, the abundance of CD45RA- IGF-1R+ naive T cells is similar in peripheral blood from control donors and those with GD. Expression of the receptor by GD-derived T cells has functional consequences in that IGF-1 enhances BrdU incorporation and inhibits Fas-mediated apoptosis (35). Herein we report that the phenotype of B cells in patients with GD is also disproportionately biased toward the CD19+ IGF-1R+ phenotype. Moreover, this skewed phenotype affects function, including enhanced B cell expansion and exaggerated Ab production. IGF-1 increases the production of IgG but not IgM in B cells derived from patients with GD compared with those from control donors. Thus, the underlying mechanism for aberrant IgG production that drives multiple aspects of GD may, at least in part, result from the overrepresentation of B cells displaying IGF-1R.

Materials and Methods

Materials

Ficoll-Hypaque was purchased from Sigma-Aldrich. Fac.lyse buffer, Cytofix, anti-CD19 CyChrome, anti-IGF-1R PE (clone 1H7), and isotype mouse IgG1 FITC, PE, allophycocyanin, and CyChrome were purchased from BD Biosciences. PBS was supplied by Invitrogen. IGF-1 and Des(1-3) IGF-1 were from Calbiochem and GroPeP, respectively.

Patient samples

Subjects, aged 20–65 years, were recruited from the patient population of Jules Stein Eye Institute and Harbor-UCLA Medical Center. Informed consent was obtained as approved by the Institutional Review Boards of the Center for Health Sciences at UCLA and Harbor-UCLA Medical Center. The study population comprised patients evaluated for GD without or with TAO. Control subjects were healthy volunteers without known autoimmune disease, asthma, granulomatous disease, sinusitis, or HIV infection. Patients with GD comprised a clinically heterogeneous group and included those who were hyperthyroid (n = 3) and euthyroid (n = 27). Twenty-eight of 30 patients manifested TAO. A minority of patients had active inflammatory disease (clinical activity score ≥3; n = 6), while most exhibited stable TAO (clinical activity score <3; n = 22). No association between IGF-1R display and disease duration or orbital inflammation was noted. Bone marrow samples were derived from a patient with GD, a healthy volunteer, or within 24 h of death. Orbital tissue was obtained from surgical waste during orbital decompression surgery in patients with TAO or from healthy individuals during cosmetic surgery. The tissue was transported on ice, homogenized, and single-cell suspensions were prepared. Tissue was filtered through 70-μm pores and processed for flow cytometry.

Clinical data including age, sex, medications, smoking history, physical exam, and laboratory values were recorded. Careful examination of the skin failed to detect evidence of thyroid-related dermopathy in any of the study participants.

Flow cytometry

Peripheral blood (−5 ml) was obtained and stored in tubes containing EDTA. Staining buffer was prepared in PBS containing 4% FBS with 0.1% sodium azide (Sigma-Aldrich). Staining for flow cytometry was performed within 24 h of blood collection, according to the manufacturer’s instructions (BD Biosciences). Briefly, 100 μl whole blood or bone marrow aspirate was placed in 12 × 75 mm polypropylene tubes and fluorochrome-conjugated mAbs were added (1 μg/10^6 cells). These were then incubated in the dark for 20 min at room temperature. FACSlyse solution (2 ml) was added for 10 min at room temperature to lyse RBCs. Cells were washed twice with staining buffer, resuspended in Cytofix (BD Biosciences), and kept in the dark at 4°C until cytometric analysis (within 24 h). Analysis was performed on a FACS caliber flow cytometer (BD Biosciences). Mean fluorescent intensity (MFI) was calculated as a ratio of mean fluorescence sample/isotype fluorescence. Percentage positive expression was determined as the population of cells with increased fluorescent intensity compared with isotype.

PBMC preparation

PBMCs were prepared using a technique described previously (36). Briefly, whole blood was diluted 1:2 in PBS and layered over Ficoll-Hypaque, centrifuged at 500 × g for 25 min, and washed three times in PBS. Cells (1 × 10^6 cells/ml) were cultured in RPMI 1640 medium supplemented with CpG (2 μg/ml) (Integrated DNA Technologies), IGF-1 (10 nM), and/or Des(1-3) IGF-1 (10 nM).

Quantification of B cell number

PBMCs were cultured as described above. An aliquot of each sample was analyzed by flow cytometry and used to calculate the fractional B cell population after live cell gate. This percentage was then multiplied by the cell number to quantify B cell number. Cell counts were determined according to the manufacturer’s protocol (CyQuant; Invitrogen). Briefly, PBMCs were cultured in 96-well plates and incubated for 24–72 h. At the desired time, the 96-well plate was centrifuged at 500 × g for 5 min and the supernatant decanted by inversion. Plates were frozen at −70°C until analysis. Culture solution did not contain phenol red since the dye can interfere with CyQuant GR dye fluorescence. Plates were thawed at room temperature and 200 μl of CyQuant GR dye/cell lysis buffer was added to each well and incubated for 5 min with gentle mixing. Samples were protected from light, and emissions were determined using a Wallac Victor 1420 fluorometer (PerkinElmer) at 480 nm excitation and 520 nm emission wavelengths. They were assayed in quadruplicate and compared with a standard curve.

B cell production of IgM and IgG

PBMCs were stimulated in vitro as previously described (37). Briefly, 2.5 × 10^6 PBMC/200 μl in 96-well plates or 5 × 10^6 PBMC/ml in 24-well plates were cultured in 10% heat inactivated FBS-supplemented RPMI 1640 for 12 days. Cells were stimulated with nothing, CpG 2006 (2 μg/ml) (Integrated DNA Technologies), Staphylococcus aureus Cowen strain I (20 μg/ml) (Sigma-Aldrich), and pokeweed mitogen (1/100,000) in the presence or absence of IGF-1 (10 nM). The chosen CpG concentration yielded substantial mitogen activity. IgM and IgG concentrations were determined after 12 days in culture. Supernatants were harvested and Ig concentrations were assayed using a commercially available ELISA, according to the manufacturer’s specifications (Bethyl Laboratories). Sample concentrations were compared with a standard curve.

PBMC preparation of anti-TSHR Abs was assayed in both CD19+ IGF-1R+ and CD19+ IGF-1R+ populations after sorting by flow cytometry (38). These cells were cultured with monocytes in a 1:2 ratio and incubated with EBV-containing supernatant generated by the B95.8 marrows cell line for 1 h at 37°C, at a concentration of 2 × 10^6 cells/ml. After EBV exposure, cells were washed and cultured at 1 × 10^6 cells/ml (RPMI 1640, 1% l-glutamine, 1% penicillin-streptomycin, and 10% FBS) in the presence of the monocytes. After 2 wk, conditioned media were analyzed for Ab according to the manufacturer’s specifications (TRAK LIA kit, BRAHMS Diagnostica).

Statistics

Values are reported as the mean ± SE. Statistical analysis was performed using a two-tailed Student’s t test with a confidence level of >95%.
Results

A greater proportion of B cells derived from patients with GD express IGF-1R compared with those from control donors

We have previously demonstrated that a disproportionate fraction of peripheral blood T cells from patients with GD express IGF-1R (35). CD4+CD45RO− and CD8+CD45RO− memory T cells from these patients displayed the greatest divergence from controls with regard to the fractional expression of IGF-1R. Given the prominent role for B cell function in the pathogenesis of GD, we have now investigated IGF-1R display by these lymphocytes in the peripheral blood and a cohort of control donors using multiparameter flow cytometry. Analogous to their T cells, B cells (CD19+, IGF-1R+) from donors with GD exhibit increased fractional expression of the receptor, as demonstrated in a representative study shown in Fig. 1. The disparity between control lymphocytes and those from the patients was similar to that found in their T cells. As shown in Fig. 2, cumulative data from 30 different patients with GD demonstrate that 34% ± 4% (mean ± SE) of B cells express IGF-1R, while the receptor was detected in 9% ± 3% cells from control donors (n = 24; p < 10−6, GD vs controls). The range of IGF-1R expression was considerably broader among patient-derived B cells (7%–98% CD19+IGF-1R+) compared with controls (1%–45%). The fraction of IGF-1R+ B cells appears durable since longitudinal examination of lymphocytes obtained serially from four different patients revealed that their abundance was invariant over a 1-year period (data not shown). Moreover, the frequency of IGF-1R+ B cells remained stable following treatment with oral prednisone in a single patient and during clinical remission in two others over 6 mo of follow-up. The fraction of IGF-1R+ B cells from patients with GD does not decline as a function of time in culture (data not shown), exhibiting similar phenotypic stability to that found previously in T cells (35).

We next investigated whether the skewed CD19+IGF-1R+ B cell phenotype found in peripheral blood from patients with GD was also present in their bone marrow and orbit. As demonstrated in Fig. 3 (upper panels), 49% of marrow-derived CD19−CD3−CD45−CD15−CD14− B cells from a single patient with GD express IGF-1R compared with 13% of cells from a control donor, representative of two control subjects (13% and 21%).}

![Figure 1](image1.png)

**FIGURE 1.** Increased fraction of peripheral blood T and B cells from a patient with GD display IGF-1R compared with those from a control donor. PBMCs were stained with anti-CD3, anti-CD19, and anti-IGF-1R Abs as described in Materials and Methods and subjected to multiparameter flow cytometry. The gray open histograms represent staining with isotype control Abs.

![Figure 2](image2.png)

**FIGURE 2.** A disproportionate fraction of peripheral blood B cells from 30 patients with GD express IGF-1R compared with that found in 24 control donors. A, Individual data sets demonstrating fractional IGF-1R+ B cells. B, Analysis of IGF-1R display in B cells as an aggregate of multiple patients with GD vs control donors (34% ± 4% IGF-1R+ B cells (mean ± SE, n = 30) vs 9% ± 3% IGF-1R+ control B cells (n = 24)). Data are expressed as means ± SE (**, p < 10−6).

![Figure 3](image3.png)

**FIGURE 3.** Increased fraction of bone marrow- and orbit-derived B cells from patients with GD express IGF-1R. Bone marrow aspirate (upper panels) from a patient with GD demonstrates increased fraction of IGF-1R+ B cells compared with a control aspirate. B cells isolated from the orbit and peripheral blood of a patient with GD (lower panels) display increased fraction of IGF-1+ B cells. Isotype Ab staining is shown as an open gray histogram. Lower right, IGF-1R expression by B cells from the peripheral blood and orbit; each sample pair was obtained from one of three separate patients.
Abundance of IGF-1R

FIGURE 4. Abundance of IGF-1R.† B cells from patients with GD is maintained or increased with CpG activation compared with those from control donors. PBMCs were isolated and cultured in the presence of CpG (2 µg/ml). IGF-1R display was assessed by flow cytometry at 24 h and is expressed as percentage change from unstimulated cultures. Each datum point represents a single patient’s sample. Broad horizontal bars indicate mean values for each group (GD vs control, p < 0.002).

Impact of IGF-1 on B cell phenotype before and following stimulation with CpG

Since IGF-1 can influence metabolic activity and proliferation of B cells (27, 39), we investigated whether it altered the expression of costimulatory molecules or cell-surface signatures associated with lymphocyte activation. As shown in Table I, basal expression of CD23, CD80, and CD86 is similar on B cells from donors with GD and their controls. As expected, CpG stimulation increased B cell expression of all three markers. In contrast, IGF-1 failed to influence levels of any of these surface molecules after 24, 48, and 72 h (48 h shown in Table I). The vast majority of B cells expressing CD23, CD80, or CD86 fail to display IGF-1R. These cells remain IGF-1R−, regardless of whether they remain untreated or are exposed to CpG, either in the absence or presence of IGF-1.

We have previously reported that expression of IGF-1R on T cells from patients with GD is aberrantly up-regulated following cell activation (35). We therefore investigated whether B cell activation was also associated with increased IGF-1R levels. CpG-provoked lymphocyte activation resulted in divergent patterns of IGF-1R display in cells from patients and their controls. This treatment either maintained or increased B cell expression of IGF-1R in most patients with GD (Fig. 4; n = 9; mean increase, 25 ± 21%). In contrast, identical treatment reduced the abundance of IGF-1R-displaying B cells from control donors (n = 5, mean decrease 64 ± 16%, p < 0.002 vs GD).

Table I. Exogenous IGF-1 does not alter surface expression of CD80, CD86, or CD23 by GD or control B cellsa

|                 | Hours in Culture | GD (n = 5) | Control (n = 5) |
|-----------------|-----------------|------------|----------------|
|                 |                 | CD221+ (%) | CD221− (%)     | Total (%) CD221+ (%) | CD221− (%)     |
| CD80†           | 0               | 6 ± 3      | 1 ± 1          | 5 ± 2                | 6 ± 3          |
| Control         | 48              | 8 ± 4      | 3 ± 2          | 5 ± 3                | 14 ± 7         | 4 ± 2          | 10 ± 5         |
| CpG             | 48              | 16 ± 5     | 4 ± 2          | 11 ± 4               | 21 ± 12        | 8 ± 4          | 20 ± 12        |
| IGF-1           | 48              | 8 ± 3      | 3 ± 2          | 8 ± 3                | 8 ± 4          | 2 ± 2          | 6 ± 4          |
| CpG + IGF-1     | 48              | 19 ± 7     | 4 ± 2          | 15 ± 6               | 15 ± 6         | 3 ± 2          | 12 ± 5         |
| CD86            | 0               | 2 ± 2      | na             | na                   | 3 ± 1          | na             | na             |
| Control         | 48              | 31 ± 7     | 9 ± 8          | 25 ± 8               | 21 ± 7         | 5 ± 1          | 17 ± 6         |
| CpG             | 48              | 65 ± 13    | 15 ± 6         | 50 ± 14              | 48 ± 13        | 8 ± 3          | 40 ± 9         |
| IGF-1           | 48              | 36 ± 16    | 7 ± 4          | 29 ± 16              | 19 ± 8         | 4 ± 1          | 15 ± 7         |
| CpG + IGF-1     | 48              | 63 ± 19    | 7 ± 4          | 55 ± 17              | 50 ± 14        | 5 ± 2          | 45 ± 9         |
| CD23†           | 0               | 17 ± 6     | 4 ± 3          | 14 ± 7               | 23 ± 11        | 6 ± 3          | 18 ± 8         |
| Control         | 48              | 19 ± 9     | 5 ± 2          | 17 ± 9               | 11 ± 5         | 3 ± 2          | 9 ± 6          |
| CpG             | 48              | 52 ± 14    | 12 ± 5         | 37 ± 10              | 54 ± 19        | 8 ± 2          | 45 ± 18        |
| IGF-1           | 48              | 21 ± 11    | 4 ± 2          | 19 ± 9               | 13 ± 7         | 3 ± 2          | 11 ± 5         |
| CpG + IGF-1     | 48              | 52 ± 18    | 5 ± 2          | 47 ± 14              | 65 ± 21        | 8 ± 3          | 57 ± 19        |

a PBMC were isolated and cultured with or without CpG (2 µg/ml) and IGF-1 (10 nM) for 48 h and assayed for expression of CD80, CD86, and CD23. Each datum point represents percentage positive-staining CD19+B cells ± SEM. All treatments compared to their respective controls were not significant. na, not applicable.
IGF-1 promotes expansion of GD-derived B cells

IGF-1 activation enhances myeloma proliferation and splenic B cell maturation in vivo but these effects in vitro have been found to be variable (40–42). We next investigated whether the increased fraction of circulating IGF-1R+ B lymphocytes found in GD conveys a functionally different cell phenotype. PBMCs or purified B cells (>95% purity) were submaximally stimulated with CpG (2 μg/ml) in the absence or presence of IGF-1 (10 nM). IGF-1 significantly enhances B cell number in cultures derived from patients with GD when added in combination with CpG compared with lymphocytes receiving CpG or IGF-1 alone (p < 0.02; Fig. 5). Moreover, Des(1–3) IGF-1 (10 nM), an analog that binds to and selectively activates IGF-1R (43), also enhances the actions of CpG and IGF-1 significantly increases IgM production only in B cells from patients with GD compared with treatment with either single agent (**, p < 0.01). Results are expressed as the means ± SE of five independent experiments.

The MFI for CD19+ IGF-1R+ B cells derived from patients and control donors was similar at all time points assayed.

FIGURE 6. IGF-1 potentiates IgM and IgG production both in B cells from patients with GD and in those from control donors. IgM and IgG were assayed after 12 days in culture stimulated with CpG and/or IGF-1. A. B. IGF-1 (10 nM) alone or in combination with CpG (2 μg/ml) significantly increase IgM production by GD and control B cells. B, CpG and IGF-1, used as single agents, enhanced IgG production by B cells from both sources (*, p < 0.05 vs unstimulated). The combination of CpG and IGF-1 significantly enhances IgG production only in B cells from patients with GD compared with treatment with either single agent (**, p < 0.01). Results are expressed as the means ± SE of five independent experiments.

IGF-1 synergistically enhances the production of IgG by B cells from patients with GD but not those from control donors

Because both autoAb production and action play central roles in the pathogenesis of GD, we next investigated whether the increased fraction of IGF-1R+ B cells in these patients might enhance Ab production. PBMCs from patients with GD and control donors were submaximally stimulated with CpG (2 μg/ml) in the absence or presence of IGF-1 (10 nM). IGF-1 significantly enhances B cell number in cultures derived from patients with GD when added in combination with CpG compared with lymphocytes receiving CpG or IGF-1 alone (p < 0.02; Fig. 5). Moreover, Des(1–3) IGF-1 (10 nM), an analog that binds to and selectively activates IGF-1R (43), also enhances the actions of CpG (data not shown; 1.4 ± 0.5-fold increase compared with CpG alone). In contrast, treatment with CpG, IGF-1, or Des(1–3) IGF-1, either as single agents or in combination, fails to expand B cells from control donors.

IGF-1 potentiates IgM and IgG production both in B cells from patients with GD and in those from control donors. IgM and IgG were assayed after 12 days in culture stimulated with CpG and/or IGF-1. A. B. IGF-1 (10 nM) alone or in combination with CpG (2 μg/ml) significantly increase IgM production by GD and control B cells. B, CpG and IGF-1, used as single agents, enhanced IgG production by B cells from both sources (*, p < 0.05 vs unstimulated). The combination of CpG and IGF-1 significantly increases IgG production only in B cells from patients with GD compared with treatment with either single agent (**, p < 0.01). Results are expressed as the means ± SE of five independent experiments.

FIGURE 6. B LYMPHOCYTE EXPRESSION OF IGF-1R IN GRAVES’ DISEASE

Discussion

Herein we report for the first time that B cells from patients with GD are overrepresented by those exhibiting the IGF-1R+ phenotype. This mirrors earlier findings by us that T cells (35) and fibroblasts (15) from these same patients also exhibit a strongly skewed phenotype with regard to cell-surface IGF-1R display. Thus, overexpression of this putative self-Ag appears to be more widely spread in GD than initially thought. The overabundance of IGF-1R+ B cells is not limited to the peripheral circulation but can also be demonstrated in affected orbital tissues from patients with TAO (Fig. 3). This receptor display imparts several functional attributes to B cell behavior in that IGF-1 enhances both survival and Ab production in vitro. Moreover, IGF-1R+ B cells can produce anti-TSHR Abs.

B cells play diverse roles in immune function by virtue of their further differentiation into Ig-secreting plasma cells and their importance in Ag presentation. A critical component of B lymphocyte development concerns how committed precursor cells from the hematopoietic lineage undergo Ig H chain gene rearrangement. This results in L chain production and leads to the release of mature B cells from the bone marrow (45). A number of factors, including IGF-1 emanating from marrow stroma, support early B cell proliferation and drive their differentiation (42, 46, 47). When administered in vivo, IGF-1 expands intrasplenic B cells through increased proliferation of mature cells (28, 48). In marrow, B cells become more abundant following IGF-1 administration in normal adult mice and also in those receiving lethal irradiation followed by reconstitution with syngeneic bone marrow (29). Additionally, IGF-1 enhances IL-7-dependent B cell proliferation in concert with kit ligand (39). Studies conducted in vitro disclose that the differentiation of human CD34+ marrow cells is impeded by down-regulating the IGF-1 produced by cocultured MS-5 cells (46). These studies implicate IGF-binding protein-6 (IGFBP-6) as a necessary component of IGF-1-dependent B cell differentiation while IGFBP-3 may act as an inhibitor. Our examination of the bone marrow from a patient with GD discloses the ubiquitous nature of IGF-1R+ B cells within this tissue and suggests the importance of the receptor and the strong survival signals it appears to convey.

IGF-1 binds peripheral human B cells selectively (49), an association that is both saturable and can be displaced with unlabelled IGF-1 and insulin. Administration of IGF-1 to mice results in elevated circulating Ab levels (28, 47). Baudler and colleagues (50) reconstituted Rag2-deficient C57BL/6 mice with fetal liver cells from IGF-1R−/− mice. T cell-independent humoral responses to the type 2 Ag, (4-hydroxy-3-nitrophenyl)acetyl-Ficoll, are substantially diminished while those against the T cell-dependent Ag, (4-hydroxy-3-nitrophenyl)acetyl-chicken globulin, appear normal. B cell development remains normal in IGF-1R-deficient chimeras.
as does T cell differentiation. In these animals, IGF-1 enhanced Ig production, an effect that proved independent of B cell proliferation (50). These earlier findings appear congruent with our own observations where incubation of B cells with IGF-1 results in a substantial increase in IgG production without concomitant changes in IgM abundance. Furthermore, Kimata et al. demonstrated that IGF-1 can enhance IgG subtype production in both tonsillar and peripheral B cells (40, 41). This effect was dependent at least in part on CD40 ligation. Our results also demonstrate that IGF-1R-displaying B cells produce activating anti-TSHR Abs and thus may participate specifically in disease pathogenesis.

IGF-1R activation results in the recruitment of docking proteins, including insulin receptor substrate (IRS) (43). This common pathway is shared by several hormone and cytokine receptors, including IL-4R, that regulate B cell Ab production (51). IRS proteins mediate IGF-1- and IL-4-stimulated activation via the PI3K signaling pathway (52). PI3K signaling positively regulates Ig production in vivo (53, 54). Increased expression of the IGF-1R may thus promote Ig production through pathways shared with IL-4R.

IGF-1 enhances DNA synthesis in myeloma cell lines in vitro in the absence of IL-6 (55). Both IGF-1 and IGF-2 up-regulate the effects of IL-6 on plasma cell proliferation. The effects are absent in normal B cells, suggesting that actions of IGF-1 may diverge in normal and malignant cells. Both IGF-1R and insulin receptors are expressed at higher levels in myeloma cell lines than in B lymphoblastoid lines (56). RPMI 8226 cells express high-affinity binding sites for both insulin and IGF-1 (57). The latter increases receptor phosphorylation, PI3K activation, DNA synthesis, and lactate production in these cells. Thus, there exists substantial precedent for IGF-1, its receptor, and the pathways it utilizes in cell signaling for regulating B cells and their plasma cell derivatives.

We have previously reported that circulating GD-IgG is directed against IGF-1R and can be detected in almost all patients with the disease, but in very few control donors (15, 16, 58). When treated with IGF-1 or with GD-IgG, cultured orbital fibroblasts from patients with GD, but not their controls, synthesize high levels of IL-16 and RANTES, two powerful T cell chemoattractants (15, 16). Pritchard, J., N. Horst, W. W. Cruikshank, and T. J. Smith. 2003. IGF-like anti-CD20 antibody therapy as a novel treatment option. Eur. J. Endocrinol. 154: 623–632.

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