Insulin-Like Growth Factor-1 Controls Type 2 T Cell-Independent B Cell Response

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The IGF-1 receptor (IGF-1R) is expressed on T and B lymphocytes, and the expression of the insulin- and IGF-1-signaling machinery undergoes defined changes throughout lineage differentiation, offering a putative role for IGF-1 in the regulation of immune responses. To study the role of the IGF-1R in lymphocyte differentiation and function in vivo, we have reconstituted immunodeficient RAG2-deficient mice with IGF-1R−/− fetal liver cells. Despite the absence of IGF-1Rs, the development and in vivo activation of B and T lymphocytes were unaltered in these chimeric mice. By contrast, the humoral immune response to the T cell-independent type 2 Ag 4-hydroxy-3-nitrophenyl acetyl-Ficoll was significantly reduced in mice reconstituted with IGF-1R-deficient fetal liver cells, whereas responses to the T cell-dependent Ag 4-hydroxy-3-nitrophenyl acetyl-chicken globulin were normal. Moreover, in an in vitro model of T cell-independent type 2 responses, IGF-1 promoted Ig production potently upon polyvalent membrane-IgD cross-linking. These data indicate that functional IGF-1R signaling is required for T cell-independent B cell responses in vivo, defining a novel regulatory mechanism for the immune response against bacterial polysaccharides. The Journal of Immunology, 2005, 174: 5516–5525.
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These data show that lymphocyte IGF-IR expression is not required for lymphocyte development and differentiation, but is specifically required for T cell-independent B cell responses. Because such T cell-independent responses represent important defense mechanisms against bacterial Ags, our findings assign IGF-1R-mediated signaling a novel role in these immune defense processes.

**Materials and Methods**

**Mice**

IGF-IR+/−, 129/Sv mice (21) were generously provided by A. Elafritiadis (Columbia University, New York, NY). RAG2-deficient mice (24) were purchased from Taconic Farms. Mice were kept in specific pathogen-free animal facilities according to institutional guidelines. DNA for typing was prepared from tail biopsies.

**Cell sorting and semiquantitative RT-PCR**

For sorting of lymphocyte subpopulations, single cell suspensions of spleen, thymus, and bone marrow of wt 129/Sv mice were prepared. According to their surface Ags, bone marrow-derived B lymphocytes were sorted into pro- and pre-B cells and immature and recirculating B cells (anti-B220/CD45R and anti-IgM) (25) splenic B lymphocytes were sorted into marginal zone (MZ) and follicular B cells (anti-B220/CD45R, anti-CD21, and anti-CD23) (26), and thymocytes were sorted into double-negative, double-positive, and CD4 and CD8 single-positive T cells (anti-CD4 and anti-CD8) (32), and isolation was confirmed using the RNAase-free DNase kit (Qiagen) according to the manufacturer’s instructions. Total RNA was treated with RNase-free DNase (Qiagen) and reverse transcribed with SuperScript II (Invitrogen Life Technologies). Twenty-five to 40 cycles of 30 s at 95°C, 45 s at 57°C (IGF-1R and IRS-2) or 60°C (GAPDH, IRS-1, and IR), and 45 s at 72°C were used for amplification using the following primers: IGF-IR 5'-5'-CAG AGG ATT GAA GGA GCC TCT CAT CTA C-3' IRS-1 5'-5'-GAG ATC TAC ACT GCA AGT GAG GCT AGA G-3'; Lambda 5'-5'-GAA GATC TAC CCT GGT GAG 3'; IR-5 5'-5'-GGC CAG AGA CAA CTA GTT ACT-3'; IRS-1 5'-5'-TGG GCA GGC ACC TTC TCA AC-3'; IRS-1 3'-5'-GGCA CAG AGG ACC AGT GAC-3'; IRS-2 5'-5'-CTT GGA GAG GAG ACT GAG GAC-3'; IRS-2 3'-5'-ATC CAT GAC GGC TAC TGT GTC-3'; GAPDH 5'-5'-ACC ACA GTC CAT GCC ATC AC-3'; and GAPDH 3'-5'-TCC ACC ACC GTG TTC GTG TA-3'. PCR products were resolved on 1% agarose gels in Tris-acetic acid-EDTA buffer, and the intensity of the bands was quantified using the QuantityOne gel documentation system (Bio-Rad).

**Analysis of protein expression and tyrosine phosphorylation**

For the analysis of tyrosine phosphorylation, MACS-purified (34) B cells were suspended in RPMI 1640 supplemented with 1% FCS and stimulated with 20 μg/ml F(ab')2 of goat anti-mouse IgM or whole goat anti-mouse IgM for the indicated time at 37°C (35). Cells were pelleted and lysed in lysis buffer (50 mM HEPES, 1 Triton X-100, 50 mM NaCl, 0.1 M sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 10 μg/ml sodium orthovanadate, 2 mM benzamidine, 0.1% SDS, and 2 mM PMSF, pH 7.4), and the lysate was clarified by centrifugation for 15 min at 12,000 × g. Fifty micrograms of protein were resolved by SDS-PAGE on 8% polyacrylamide gels and blotted on Hybond ECL nitrocellulose membranes (Amersham Biosciences). After blocking with 1× blocking reagent (Roche), membranes were incubated with mouse anti-phosphotyrosine (PY99, Santa Cruz Biotechnology), Akt, or pAkt (Cell Signaling Technology and New England Biolabs) mAb and peroxidase-conjugated anti-mouse or anti-rabbit IgG (Sigma-Aldrich). Bound Ab was then detected by ECL (Amersham Biosciences). For analysis of IGF-1R and IR protein expression, whole embryos were homogenized in lysis buffer, and lysates were processed as described above. After protein transfer, nitrocellulose membranes were incubated with anti-mouse IGF-1Rβ (C-20) or IRβ Ab (C-19, Santa Cruz Biotechnology) and HRP-conjugated anti-rabbit IgG (Sigma-Aldrich).

**Analysis of B cell proliferation and up-regulation of activation markers**

Splenocytes derived from chimeras were cultivated at 1.5 × 10^6 cells/well in 96-well plates and were stimulated with F(ab')2 of goat anti-mouse IgM or whole anti-mouse IgM (2.5 μg/ml; DianoVa), hamster anti-mouse Cd40 Ab (2 μg/ml) (BD Pharmingen), mouse rIL-4 (25 μU/ml; R&D Systems), LPS from Escherichia coli serotype 055:B5 (20 μg/ml; Sigma-Aldrich), and human rIGF-1 (10 μg/ml; Sigma-Aldrich) in RPMI 1640 medium. After 48 h, cells were harvested and stained with PE- or FITC-conjugated Abs to CD4, CD8, B220/CD45R (RA3-6B2), and F(ab')2 of goat anti-mouse IgM, anti-B220/CD45R (RA3-6B2) to gate on B cells, CD86 (B7.2; BD Pharmingen) and human rIL-4, and MHCII (M5/114; BD Pharmingen) to assess the activation status (35).

For determination of proliferation, splenic B cells were purified by depletion of non-B cells with anti-Cd43 Ab coupled to magnetic beads and MACS columns (Miltenyi Biotech) as described previously (34), plated on 96-well plates at 10^5 cells/well and stimulated with 0.1, 1, or 10 μg/ml F(ab')2 of goat anti-mouse IgM, hamster anti-mouse Cd40 (36), or LPS and 0.1, 1, or 10 ng/ml dextran-coupled anti-IgG alone or in combination with IL-4 (25 μU/ml), IL-5 (0.1 ng/ml), or IGF-1 (10−7 M) for 72 h and measured in a CTG-160 medium supplemented with 10% FCS (35, 37). After addition of 0.5 μCi [methyl-3H]thymidine/well (Amersham Biosciences), cells were incubated for 12–16 h, harvested with a cell harvester (Packard Instruments), and bound to a filter. Incorporation of [3H]thymidine was measured in a beta counter (Beckman Coulter). All experiments were performed in triplicate.

**Immunization of recombinant mice**

The ability of chimeric mice to mount a humoral immune response was assessed by immunization of IGF-1R+/− and wt recombinant mice i.p. with the hapan 4-hydroxy-3-nitrophenyl acetyl (NP) (38). To assess the immune response to a T cell-independent Ag, mice received 10 μg of NP-BSA in PBS; to assess the T cell-dependent primary immune response, mice received 100 μg of alumn-precipitated NP17-chicken globulin (39) i.p.; for the secondary response, mice were reimmunized i.p. with 10 μg of soluble NP-BSA on day 42 after the first immunization. Sera were collected on days 0, 7, 14, 21, and 28. To detect the presence of NP-specific Abs, ELISA plates were coated with 5 μg/ml NP-BSA and blocked with 10^6 cells/10 μl in staining buffer (PBS containing 0.5% BSA and 0.01% NaN3) with optimal amounts of FITC-, PE-, biotin-, CyChrome-, or allophycocyanin-conjugated Abs. Abs were purchased from BD Pharmingen and Serotec; flow cytometric analysis was performed on a FACSCalibur cytometer (BD Biosciences).

**Fetal thymic organ culture**

IGF-IR+/− mice were mated, and the occurrence of vaginal plugs was defined as embryonic day (ED) 0.5. Embryos were prepared on ED 14.5–17.5, and fetal thymus were cultured up to 1 wk on Milliculture plate inserts (Millipore) in DMEM-10 (DMEM; Life Sciences; 10% FCS, 6 ml of sodium pyruvate, 6 ml of nonessential amino acids, 6 ml of glutamine, and 50 μM 2-ME) at 37°C in 7.5% CO2 and 100% relative humidity. For FACS analysis, thymic lobes were passed through a 30-μm pore size nylon mesh. Potentially migrated thymocytes were collected by centrifugation of the culture medium from the respective wells and rinsing the plate culture inserts with PBS. Cells were stained with anti-Cd4 and anti-Cd8 Abs as described above.

**Analysis of proliferation and migration**

For determination of proliferation, splenic B cells were purified by depletion of non-B cells with anti-Cd43 Ab coupled to magnetic beads and MACS columns (Miltenyi Biotech) as described previously (34), plated on 96-well plates at 10^5 cells/well and stimulated with 0.1, 1, or 10 μg/ml F(ab')2 of goat anti-mouse IgM, hamster anti-mouse Cd40 (36), or LPS and 0.1, 1, or 10 ng/ml dextran-coupled anti-IgG alone or in combination with IL-4 (25 μU/ml), IL-5 (0.1 ng/ml), or IGF-1 (10−7 M) for 72 h and measured in a CTG-160 medium supplemented with 10% FCS (35, 37). After addition of 0.5 μCi [methyl-3H]thymidine/well (Amersham Biosciences), cells were incubated for 12–16 h, harvested with a cell harvester (Packard Instruments), and bound to a filter. Incorporated [3H]thymidine was measured in a beta counter (Beckman Coulter). All experiments were performed in triplicate.
3% BSA. Sera were added at various dilutions. For the T-independent immune response, IgM, IgG1, IgG3, and \( \alpha/\beta \)-bearing Ab levels were determined, and for the T-dependent response, IgG1 and IgG2a, IgG2b, IgG3, and IgA were assessed using a biotin/alkaline phosphatase-coupled streptavidin system (Roche). Additionally, basal levels of IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA were measured.

**In vitro Ab secretion**

To assess the secretory response of B cells to TI-2 Ag, splenic B cells derived from C57BL/6 mice were plated at 5 \( \times \) 10^5 cells/well in 96-well plates and stimulated with dextran-coupled anti-IgD (10 ng/ml) (37), IL-4 (25 U/ml), IL-5 (0.1 ng/ml), and IGF-1 (10^{-7} M) alone or in combination for 7 days in RPMI 1640 medium supplemented with 10% FCS. After collection of the supernatants, Ig levels were determined by ELISA using a biotin/alkaline phosphatase-coupled streptavidin system as described above.

**Immunohistochemistry**

Cryosections (6 μm) of spleens from reconstituted mice were stained for MZ B cells using rat anti-mouse mucosal addressin cell adhesion molecule-1 (MadCAM-1) Ab (Southern Biotechnology Associates) (40).
Briefly, sections were fixed in acetone, and endogenous peroxidase was blocked with H2O2 in PBS and 0.5% BSA. Blocking was performed with 10% FCS in PBS. Bound anti-MadCAM-1 was detected with peroxidase-conjugated goat anti-rat IgG and diaminobenzidine.

Results

Expression of insulin signaling components during murine lymphocyte development

Because previous studies have analyzed the expression of the IGF-1R during lymphocyte development mainly in human cells, we first determined the expression of insulin signaling components during murine lymphoid development. Bone marrow-derived cells of two groups of three wt 129/Sv mice were FACS-sorted into pro- and pre- (IgM^high^H11002 B220^int^), immature (IgM^high^B220^int^), and recirculating B cells (IgM^high^B220^high^). Total RNA was isolated and expression of IGF-1R, IR, IRS-1, and IRS-2 mRNA was determined by RT-PCR using gene-specific primers. This analysis revealed that expression of the IGF-1R is highest in the pro/pre-B cell fraction, but decreases during B lymphocyte differentiation in bone marrow. In contrast, IR and IRS-2 expression show an increase during B lymphocyte differentiation, whereas IRS-1 mRNA is detectable at the highest degree in the pro/pre-B fraction and steadily declines during further B cell differentiation (Fig. 1).

To verify the purity of the sorted B cell subpopulations, PCRs for TdT, A5 and a 0.8-kb sterile transcript from the κ L chain locus were performed on the cDNAs from pro- and pre-, immature and mature B cells. This analysis confirmed the successful separation into the respective subpopulations.

The analysis of B cells derived from spleen showed expression of IGF-1R in MZ (B220^high^CD21^high^CD23^low^) and follicular (B220^high^CD21^high^CD23^high^) B cells as well as expression of IR mRNA. Although IRS-1 expression was hardly detectable in both splenic B cell populations, IRS-2 mRNA expression was evident in follicular and MZ B cells (Fig. 1).

To determine the expression of insulin signaling components during T cell development, total RNA was extracted from FACSPurified thymocytes. Expression analysis revealed expression of the IGF-1R in double-negative (CD4^−^CD8^−^), double-positive (CD4^+^CD8^−^), CD4 single-positive (CD4^+^CD8^−^), and CD8 single-positive (CD4^−^CD8^+^) cells. IR mRNA expression was lowest in double-negative T cells and peaked at the double-positive stage, and CD4 single-positive T cells showed a greater expression of IR
mRNA than CD8 single-positive cells. Compared with this, IRS-1 and IRS-2 mRNAs were only weakly expressed in thymocytes (Fig. 1).

Taken together, this analysis revealed that IGF-1R is the pre-dominantly expressed receptor tyrosine kinase of the insulin family in the main lymphoid compartments, suggesting specific functions of the IGF-1R signaling machinery in lymphocytes.

**Generation of chimeric mice lacking functional IGF-1R expression in lymphocytes**

To generate mice with IGF-1R−/− T and B cells, IGF-1R−/− mice were mated, and embryos were delivered by cesarean section on day 16.5 postconception. Embryos were genotyped by PCR and 10⁶ FLC derived from homozygous IGF-1R−/− or wt littermates were injected i.v. into irradiated recipient animals. Western blot of tissue lysates of the same embryos verified the efficient inactivation of the IGF-1R gene. Expression of the related IR was unaltered (Fig. 2A).

Five weeks after FLC transfer, blood was collected from the tail tip, and reconstitution was verified by staining B and T cells. Mice reconstituted with IGF-1R−/− and wt FLC showed similar distribution of blood B and T cells (data not shown), whereas in unreconstituted RAG2-deficient mice, no B and T lymphocytes could be detected (data not shown). Six weeks after transfer, reconstituted mice were killed, and primary and secondary lymphoid organs were isolated. Spleen, thymus, and lymph nodes from IGF-1R−/− and wt reconstituted mice were similar in size and morphology, and cell counts of these organs and of cells derived from bone marrow and peritoneal cavity did not show significant differences (Fig. 2B).

**Normal B cell development in IGF-1R-deficient chimeras**

Previous reports have indicated a role for IGF-1 signaling in lymphocyte development, so cells from lymphoid organs were analyzed by flow cytometry to estimate the influence of IGF-1R deficiency on lymphocyte differentiation. Cells from the bone marrow of IGF-1R−/− and wt reconstituted mice did not show significant differences in the proportions of pro-B cells (IgM−B220lowCD43−), pre-B cells (IgM−B220intCD43+), immature B cells (IgMhighB220intCD43−), and recirculating B cells (IgMhighB220highCD43+; Fig. 3). The amounts of immature IgMhighIgDlow and mature IgMlowIgDhigh B cells in spleen (Fig. 3) and lymph nodes (not shown) were also similar. Moreover, we detected no difference in the expression of CD19, heat-stable Ag, and the FcγRI CD32 (not shown). Taken together, we conclude...
that B lymphocyte development occurs independently of IGF-1R expression on B cells.

Unaltered T cell differentiation in IGF-1R-deficient mice

T cell differentiation in wt and IGF-1R−/− reconstituted chimeras was assessed by staining of thymocytes with anti-CD4 and CD8 Abs. Both groups of mice showed similar proportions of double-negative (CD4−CD8−), double-positive (CD4+CD8+), and CD4 (CD4+CD8−) and CD8 (CD4−CD8+) single-positive T cells (Fig. 4A). Moreover, numbers of CD4 and CD8 single-positive T cells in spleen and lymph nodes were comparable in these mice, and there were no differences in the expression of CD3ε, TCR αβ, CD44, or the IL-2R α-chain CD25 (not shown). Therefore, T cell autonomous expression of IGF-1R is not required for T cell differentiation.

Previous experiments have indicated a critical role for IGF-1R in T cell differentiation, especially in the transition from the CD4−CD8− double-negative to the double-positive state, which could be blocked by anti-IGF-1R Abs (15). By contrast, in our analyses of FLC reconstituted mice we could not demonstrate a role for lymphocyte autonomous IGF-1R expression in T cell development. Proper T cell differentiation is thymic epithelium dependent (41, 42), and in the reconstitution model, thymic epithelium is host derived. Hence, the influence of stromal IGF-1R deficiency on T cell differentiation cannot be estimated. To address this issue, we decided to study T cell development in FTOC. We cultured thymi isolated from IGF-1R-deficient embryos and control littermates on ED 16.5 and performed FACS analysis of CD4 and CD8 expression after 6 days in culture. These analyses revealed unaltered T cell differentiation in the absence of functional IGF-1R expression (Fig. 4, B and C). These data provide clear evidence that nonlymphocyte autonomous IGF-1R expression is not required for thymocyte differentiation.

In vitro B cell activation and protein tyrosine phosphorylation

After stimulation of the BCR, B cells are activated, and an intracellular phosphorylation cascade is initiated. To elucidate the role of IGF-1R expression in the ability of B cells to mount such responses, we purified splenic B cells and induced intracellular tyrosine phosphorylation by cross-linking surface IgM by goat anti-mouse IgM. The pattern of phosphorylated proteins recognized by PY99 Ab was similar in IGF-1R-deficient and wt B cells (Fig. 5A). Analysis of intracellular signaling cascades, such as the activation of protein kinase B/Akt (Fig. 5A) and JNK (not shown), also showed no difference. These results indicate that B
cells from IGF-1R−/− chimeras retain functional signaling capacities after BCR activation, and consequently, BCR-activated signaling occurs independently of IGF-1R expression.

To further address the ability of IGF-1R-deficient B cells to undergo activation, we determined the expression of CD86 in response to B cell activation. Cross-linking of IgM, CD40 (Ab-mediated), or CD14 (with LPS from E. coli) led to increased expression of CD86 (B7.2; Fig. 5B) and MHC class II (not shown) on the surface of IGF-1R−/− B cells, and this increase was comparable to that seen in cells derived from control mice. Along this line, the proliferative response of splenic B cells to BCR cross-linking and CD40 stimulation over a range of different concentrations was also unaltered in the absence of functional IGF-1R expression (Fig. 5C).

**Decreased T cell-independent B cell response in IGF-1R−/− chimeric mice**

To determine whether chimeric mice develop functional lymphocyte subpopulations and are able to mount normal immune responses, mice were immunized with a T cell-dependent (NP-CG) (39) or T cell-independent (NP-Ficoll) Ag (38). Before immunization, sera derived from both groups of mice showed similar levels of Ig isotypes (Fig. 6A). Astonishingly, after immunization with the T cell-independent Ag NP-Ficoll, serum levels of NP-specific IgM, IgG1, IgG3, and γ-bearing Abs were determined in four mice per group on days 7, 14, 21, and 28 after immunization. C, NP-specific IgG1 and γ-bearing Abs in the sera of mice immunized with NP-CG as T cell-dependent Ag. For determination of the primary immune response, sera of three or four mice per group were collected 7, 14, 21, and 28 days after immunization. For the secondary immune response, the same mice were reimmunized i.p. on day 42 after the first immunization, and sera were collected on days 7 and 14.

**IGF-1 promotes Ig production in an in vitro model of TI-2 response**

Because mice reconstituted with IGF-1R−/− FLC showed decreased Ab production in response to TI-2 Ag NP-Ficoll in vivo, although MZ and B-1 B cell populations were normal, we decided...
to investigate whether IGF-1 is able to promote Ig production in an in vitro model for TI-2 responses. Previous studies have demonstrated that multivalent cross-linking of membrane IgD and specific costimulation with IL-4 and IL-5 serve as such a model for TI-2 responses (46, 47). Therefore, we incubated purified splenic B cells from wt C57BL/6 mice with a dextran-coupled anti-IgD mAb (H9251/H9254-dex), IL4, IL-5, and IGF-1, alone or in combination (Fig. 8). This analysis revealed that in combination with multivalent IgD cross-linking and IL-5, IGF-1 greatly increases IgM secretion in B cells, and this response significantly surmounts the response to H9251/H9254-dex with either cytokine alone. To test whether this is due to IGF-1-stimulated proliferation of these cells, we performed B cell proliferation assays under the same conditions. This analysis revealed that IGF-1 did not enhance H9251/H9254-dex-stimulated cell cycle progression. We conclude that IGF-1 specifically enhances Ig production, and that this effect is not due to IGF-1-induced B cell proliferation.

Discussion

Signaling through receptors of the IR tyrosine kinase family has been implicated in the regulation of multiple functions in the immune system, particularly in the development and differentiation of T cells. The results of the present study indicate that in vivo, IGF-1R expression on lymphocytes is not required for their differentiation, because in analyses of IGF-1R−/− mice, we could not find any defects in the differentiation of T and B cells.

Differentiation of lymphocytes is highly dependent on contact with stromal cells of the respective lymphoid organs (41, 42, 48, 49), and in the reconstitution model, such stromal cells derive from the FLC recipient, hence bearing the genotype of the host. In view of previous studies demonstrating a block of T cell development by incubation of FTOC with a blocking anti-IGF-1R Ab (15), we investigated whether IGF-1R expression on thymic epithelial cells might be required for T cell differentiation. Therefore, we cultured fetal thymi derived from IGF-1R−/− embryos and control littersmates. In this analysis we also could not demonstrate disturbed T cell development in the absence of functional IGF-1Rs. These findings underline the importance of genetic approaches to inactivate genes in vivo to address the importance of gene products in biological processes. Taken together, our data clearly rule out an important role for T cell autonomous and nonautonomous IGF-1R expression in T cell differentiation.

Surprisingly, despite normal B lymphocyte differentiation and activation in in vitro experiments, immunization of IGF-1R−/− reconstituted mice resulted in reduced Ag-specific Ig production in response to a T cell-independent Ag, whereas responses to a T cell-dependent Ag were normal. We provide two lines of evidence for a role of IGF-1 signaling in the regulation of TI-2 immune responses: 1) decreased generation of Ig in response to a TI-2 Ag in IGF-1R−/− chimeras in vivo, and 2) the ability of IGF-1 to enhance Ig production after treatment of B cells with a TI-2-like stimulus.

After ligand binding, IGF-1Rs recruit and phosphorylate IRS proteins. This mechanism is not unique for the IGF-1R, but is also common for other hormone and cytokine receptors, namely the IL-4R. Previous studies have provided a specific role for IL-4 in mediating TI-2 responses. Given that on a molecular level IGF-1 and IL-4 signaling pathways converge, it is tempting to speculate that growth factor and cytokine use the same mechanism to regulate the TI-2 immune response. This is supported by our finding.
that IGF-1 could enhance α6-dex- and IL-5-stimulated IgM production in purified B cells as potently as IL-4.

Furthermore, IRS proteins are the mediators for both IGF-1- and IL-4-stimulated activation of the PI3K pathway (50, 51). Interestingly, it has been demonstrated that PI3K signaling positively regulates TI-2 responses in vivo; thus, impaired IGF-1R signaling and consequently reduced PI3K activation could lead to a disturbed TI-2 response.

Another substrate of the IGF-1-R is the Grb2-associated binder 1 (Gab-1), that is tyrosine phosphorylated upon IGF-1 binding (52). It has been shown that a deficiency in Gab-1 causes an increased Ig secretory response after stimulation with α6-dex Abs or TI-2 immunization of chimeric mice lacking Gab-1 in B and T cells (53). This study demonstrated that the ability of Gab-1 to recruit Src homology region 2 domain-containing phosphatase 1 is required for its inhibitory role in TI-2 responses. Our previous work showed that Gab-1 overexpression in IRS-1-deficient embryonic fibroblasts strongly enhanced activation of the MAPK pathway (52). Thus, Gab-1 and IRS-2 could act as competitive substrates for the IGF-1-R and, as outlined above, potentially the IL-4-R. Gab-1 deficiency might thereby enhance the ability of IGF-1 to activate the PI3K pathway and consequently TI-2 responses.

Given the convergence of IGF-1 and IL-4 signaling pathways at the level of IRS proteins, genetically determined insulin resistance, as present in patients suffering from type 2 diabetes mellitus, might also influence the ability of IGF-1 and insulin to regulate TI-2 responses in MZ B cells. Along this line, it is clinically well established that type 2 diabetics are poor responders to pneumococcus vaccination, the clinical correlate of the TI-2 immune response. Therefore, insulin-sensitizing drugs might improve this scenario, beyond their direct effect on the improvement of glucose metabolism. Taken together, the present study assigns IGF-1 a cytokine-like action in the control of the immune response against bacterial polysaccharides. Additional dissection of the intracellular signaling pathway used will potentially define a novel target for improved vaccination strategies in diabetic patients.

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Disclosures

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