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Insulin-Like Growth Factor-I Regulation of Immune Function: A Potential Therapeutic Target in Autoimmune Diseases?

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***Abstract*——This topically limited review explores the relationship between the immune system and insulin-like growth factors (IGF-I and IGF-II) and the proteins through which they act, including IGF-I receptor (IGF-IR) and the IGF-I binding proteins. The IGF/IGF-IR pathway plays important and diverse roles in tissue development and function. It regulates cell cycle progression, apoptosis, and the translation of proteins. Many of the consequences ascribed to IGF-IR activation result from its association with several accessory proteins that are either identical or closely related to those involved in insulin receptor signaling. Relatively recent awareness that IGF-I and IGF-IR regulate immune function has cast this pathway in an unexpected light; it may represent an important switch governing the quality and amplitude of immune responses. IGF-I/IGF-IR signaling may also participate in the pathogenesis of autoimmune diseases, although its relationship with these processes seems complex and relatively unexplored. On the one hand, IGF-I seems to protect experimental animals from developing insulin-deficient diabetes mellitus. In contrast, activating antibodies directed at IGF-IR have been detected in patients with Graves' disease, where the receptor is overexpressed by multiple cell types. The frequency of IGF-IR$^+$ B and T cells is substantially increased in patients with that disease. Potential involvement of IGF-I and IGF-IR in the pathogenesis of autoimmune diseases suggests that this pathway might constitute an attractive therapeutic target. IGF-IR has been targeted in efforts directed toward drug development for cancer, employing both small-molecule and monoclonal antibody approaches. These have been generally well-tolerated. Recognizing the broader role of IGF-IR in regulating both normal and pathological immune responses may offer important opportunities for therapeutic intervention in several allied diseases that have proven particularly difficult to treat.**

I. Introduction

Insulin-like growth factors (IGF-I[1] and IGF-II), their binding proteins (IGFBPs), and the receptors mediating their signaling (types I and II IGF-IR), play critical roles in normal development, growth, metabolism, and homeostasis (Adams et al., 2000; De Meyts and Whittaker, 2002). The IGF-I pathway exerts such diverse influence on mammalian biology that the scope of its function is only now beginning to be understood. It has been insinuated in fundamental processes such as determining life span and coping with oxidative stress in rodents (Holzenberger et al., 2003). IGF-IR bears both structural and functional resemblance to other closely related tyrosine kinase receptors, such as InR in *Drosophila melanogaster* (Kennington et al., 2006) and DAF-2 in *Caenorhabditis elegans* (Kenyon et al., 1993; Dorman et al., 1995; Kennington et al., 2007). It begins functioning during fetal development and retains its importance throughout life, although the consequences of its normal or abnormal activation change with aging. IGF-IR and its related proteins have been implicated in many diseases, including growth abnormalities, metabolic disorders, and several forms of cancer (Baserga et al., 2003; Kant et al., 2007; Frasca et al., 2008). Thus, this pathway continues to attract interest as a potentially useful target for therapeutic design (Clemmons, 2007).

Detection of IGF-I and IGF-IR mRNAs and the proteins they encode in peripheral blood mononuclear cells suggests that this pathway might serve some regulatory function in the "professional" immune system. Moreover, IGF-I production, action, and intracellular signaling can be influenced by multiple cytokines and the pathways they use. IGF-IR expression on the surface of T lymphocytes can be down-regulated after cell activation (Schillaci et al., 1998). IGF-I enhances diverse aspects of bone marrow function, including lymphocyte maturation (Clark et al., 1993), granulopoiesis (Merchav et al., 1988), and erythropoiesis (Kurtz et al., 1982). Growth hormone (GH), which drives much of the IGF-I generation occurring in liver, promotes hematopoietic growth (Murphy et al., 1992a,b,c). Its effects are substantial in that they can attenuate the myelosuppressive effects of powerful chemotherapeutic agents such as azidothymidine (Murphy et al., 1992a,b,c). Administration of GH and IGF-I or driving the production of IGF-I and IGF-II using transgenic approaches in animals promotes both B and T cell development. Thus, there is reason to explore the potential for this endocrine pathway as a regulator of immunity. Moreover, targeting IGF-I and IGF-IR signaling as a strategy for altering the natural course of chronic inflammation may become an attractive means of managing autoimmune disease.

This review attempts to describe recent findings implying that the IGF-I/IGF-IR pathway plays diverse roles in regulating immune function. These new insights become particularly important in the context of therapy

[1]Abbreviations: A23187, calcimycin; Akt/PKB, protein kinase B; AP-2, adaptor protein-2; CP-751,871, figitumumab; CRP, C-reactive protein; DM, diabetes mellitus; EAE, experimental autoimmune encephalomyelitis; EGFR, epidermal growth factor receptor; EH, Eps homology; EHD, Eps homology domain; Eps, epidermal growth factor receptor pathway substrate; Erk, extracellular signal-regulated kinase; GD-IgG, Graves' disease-related immunoglobulin G; GH, growth hormone; Ig, immunoglobulin; IGF, insulin-like growth factor; IGFBP, insulin-like growth factors binding protein; IGF-IR, insulin-like growth factor-I receptor; IL, interleukin; IMC-A12, cixutumumab; IR, insulin receptor; IRS, insulin receptor substrate; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3, phosphatidylinositol 3; PKB, protein kinase B; RA, rheumatoid arthritis; RANTES, regulated upon activation, normal T-cell expressed; RXR, retinoid X receptor; SH-2, *Src* homology 2; Shc, *Src* homologous and collagen protein; SHP-2, SH-2-containing phosphotyrosine phosphatase-2; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription; TAO, thyroid-associated ophthalmopathy; TCR, T cell receptor; TNF, tumor necrosis factor; TSHR, thyrotropin receptor or thyroid-stimulating hormone receptor.

discovery. A number of biological agents, both small molecules and monoclonal antibodies, are entering the late stages of development. They have been examined as potential treatment for neoplastic diseases (Baserga et al., 2003; Clemmons, 2007). The widening scope of activities recently ascribed to IGF-I should provoke a search for broader applications for agents that can disrupt IGF-IR signaling through a variety of mechanisms. If IGF-I/IGF-IR regulates immune function, autoimmune diseases might represent unanticipated indications for drugs targeting this pathway.

II. Structure and Biology of Insulin-Like Growth Factor-I, Insulin-Like Growth Factor Receptor, and Insulin-Like Growth Factor-I Binding Proteins

A. Insulin-Like Growth Factor-I

IGF-I represents one of several structurally related polypeptides that also include IGF-II, insulin, and relaxin (Bryant-Greenwood and Schwabe, 1994). It comprises 70 amino acids organized into A and B chains connected by disulfide bonds. (Fig. 1) The amino acid sequence of human IGF-I was first reported by Rinderknecht and Humbel (1978). IGF-I possesses a connecting or C-peptide region of 12 amino acids. This region has been shown to determine the high-affinity binding of IGF-I to the type I IGF-IR (Bayne et al., 1989). An eight-amino acid D-region peptide forms an extension of the carboxyl terminus (Brissenden et al., 1984).

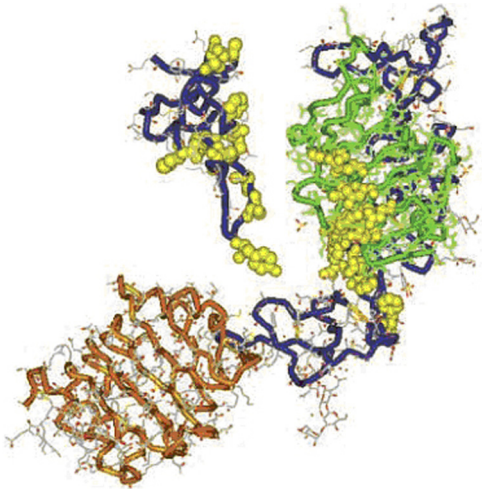

FIG. 1. Structures of the IGF-I receptor and its ligand. Three-dimensional structure of large domain 1 (L1)–Cys-rich (CR)-L2 domain of IGF-IR determined by X-ray crystallography. An extended bilobed structure ($40 \times 48 \times 105$ Å) comprises the two globular L-domains with a new type of right-handed β-helix fold that flanks the CR domain. They seem to be part of the leucine-rich-repeat superfamily. Although L1 (residues 1–15; green) contacts the CR domain (blue) along its length, there is minimal contact with L2 (residues 300–460; orange). Flexibility between CR domain and L2 could affect ligand binding. A ~30-Å diameter cavity represents a potential binding pocket. The amino acids that have been determined by alanine-scanning mutagenesis to be important for ligand binding are shown in yellow as van der Waals spheres. Three-dimensional IGF-I structure is based on the X-ray coordinates from Brzozowski et al. (2002). The backbone is shown in blue. [Reproduced from De Meyts P and Whittaker J (2002) Structural biology of insulin and IGF1 receptors: implications for drug design. *Nat Rev Drug Discov* **1**:769–783. Copyright © 2002 Nature Publishing Group. Used with permission.]

IGF-I and IGF-II circulate in the plasma as complexes formed with IGFBPs that apparently serve several biological functions. The vast majority of IGF-I (99%) is bound to IGFBP3 or IGFBP5 and is coupled with a glycoprotein called the acid labile subunit (Baxter, 1993; Twigg and Baxter, 1998). However, the full repertoire of biological implications ascribed to "free" versus bound IGF-I has yet to be determined.

Two distinct tissue sources of IGF-I production separate its functions. First, the liver generates IGF-I, which acts as an extension of the GH axis by virtue of tonic pituitary stimulation of hepatic synthesis (Maiter et al., 1988). In fact, GH, in concert with nutritional factors, represents the major determinant of circulating IGF-I levels (Oster et al., 1995). In this regard, IGF-I functions in a manner characteristic of other endocrine pathways. Second, IGF-I is also produced locally by many peripheral cell types under basal conditions and in response to inflammatory cues. In this case, IGF-I acts on peripheral tissues as an autocrine or paracrine factor resembling cytokines and other growth factors.

Whatever the source of IGF-I, responses to it are frequently mediated through the moderately high-affinity association it displays for IGF-IR (LeRoith et al., 1995; Adams et al., 2000). In other situations, members of the IGFBP family bind IGF-I, in some cases at higher affinities than those occurring with IGF-IR. IGF-I/IGFBP complex formation can produce signal initiation. Alternatively, formation of these complexes can limit the occupation by IGF-I of IGF-IR and impose limits on cellular responses dependent on IGF-IR activation. Thus, IGFBPs, IGF-I, and IGF-IR form an important pathway that can exert substantial self-regulation and can form endocrine, paracrine, and autocrine loops through which these molecules exert their biological impact. A number of factors influence the turnover of IGFs. Among these are the relative levels of both proteases and protease inhibitors found in the microenvironment of target cells. Proteases directed at IGF-I and specific IGFBPs can promote the degradation and clearance of the growth factor (Roth et al., 1984; Bhaumick and Bala, 1987; Misbin and Almira, 1989; Cwyfan Hughes et al., 1992; Myers et al., 1993; Timmins et al., 1996; Skjaerbaek et al., 1998). Some of these proteases are regulated by IGF-I itself (Myers et al., 1993). Modulation of these enzymes must then be considered potentially important determinants of the biological activity of IGF-I. The IGF-I structural variant, IGF-I Des 1–3, can also be generated as a consequence of proteolytic digestion (Maake et al., 1997). This analog represents an IGF-IR-specific activator that lacks the N-terminal three amino acids (Bagley et al., 1989; Ross et al., 1989; Yamamoto and Murphy, 1995; Jansson et al., 1997). It exhibits high affinity for the type I receptor but does not bind

or activate the IGFBPs. Moreover, it is more active than IGF-I in terms of its receptor-dependent signaling (Jansson et al., 1997). The proteolytic fragmentation of IGF-I into Des 1–3 seems to be regulated in part by the serine protease inhibitor Spi 2.1, which is down-regulated in GH-deficient rodents (Maake et al., 1997). This finding suggests a potential mechanism for regulating the availability of IGF-I in GH deficiency.

B. Insulin-Like Growth Factor-I Receptors

Type I IGF-IR consists of 1368 amino acids (Fig. 2) and belongs to a family of relatively large transmembrane tyrosine kinase receptors. These include the insulin receptor (IR) and a third, orphan member, namely IR-related receptor, the endogenous ligand for which has yet to be identified (LeRoith et al., 1995). These proteins share considerable structural similarities (Lawrence et al., 2007). The extracellular domain of IGF-IR, which is the site of constitutive receptor dimerization, can be subdivided into six protein domains. These include two L domains (L1 and L2) located in the N terminus, a cysteine-rich domain, and three fibronectin domains, termed FnIII. The second of the fibronectin domains contains a cleavage site between residues 708 and 710. Cleavage at this site results in the formation of two polypeptides, IGF-IRα and IGF-IRβ, that are linked by disulfide bonds. The residues determining IGF-I and IGF-II binding apparently reside in the L1 and second FnIII domains (Whittaker et al., 2001; Sørensen et al., 2004). IGF-IR was established as a protein distinct from IR some 35 years ago (Megyesi et al., 1975). Its critical importance to normal development and physiology is underscored by the neonatal lethality resulting from a complete absence of IGF-IR (Liu et al., 1993). On the other hand, after the conclusion of linear growth in mammals, its functions seem less critical. At that stage, IGF-IR serves other important functions, such as secondarily regulating carbohydrate metabolism and perhaps influencing immune function. Among the potential ligands, it binds IGF-I with the highest affinity but also displays appreciable avidity for IGF-II and insulin. The affinities for these latter two ligands are 1 and 2 orders of magnitude lower, respectively, than the affinity for IGF-I. Type II IGF-IR, which has been shown to be identical to the cation-independent mannose 6 phosphate receptor (Kornfeld, 1992; Hassan, 2003), binds IGF-II with the greatest avidity but can also bind IGF-I. Unlike the type I receptor, type II IGF-IR fails to bind insulin. Its signaling potential is considered relatively minor. Rather, it may function to promote ligand clearance. It represents a single membrane-spanning domain-containing glycoprotein (Ghosh et al., 2003). The extracellular domain comprises 15 cysteine-rich repeats, whereas the carboxyl terminus is quite short. Two distinct binding sites accommodate IGF-II and mannose-6-phosphate (Braulke, 1999). Type I and II receptors may mediate Erk 1/2 phosphorylation provoked by IGF-I and IGF-II, respectively (El-Shewy et al., 2007). By knocking down type I IGF-IR, the activation of Erk p42/44 elicited by IGF-I is substantially abrogated, whereas that of IGF-II persists. In contrast, interfering with the type II receptor had little effect on IGF-I signaling to Erk, whereas the activities of IGF-II on the activation of this kinase were reduced. Thus, type I and II receptors might function independently with regard to Erk activation, and IGF-II might exert at least some of its actions through the type II receptor (El-Shewy et al., 2007). The signaling initiated through IGF-IR begins with a conformational change provoked by receptor ligation and involves a number of well used pathways in tissues in which IGF-I exerts its actions. In solution, type I IGF-IR can bind three molecules of IGF-I (Whitten et al., 2009). Moreover, binding of the ligand to this receptor results in relatively little structural movement and may be limited to local rotation of protein domains.

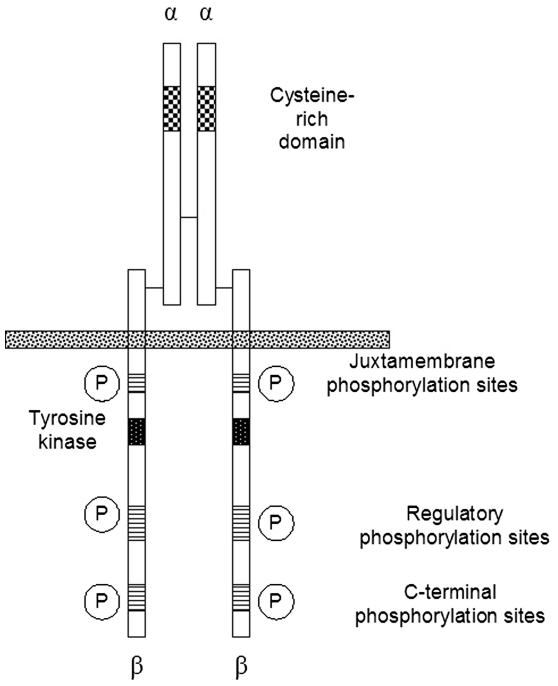

FIG. 2. Schematic of the IGF-IR dimer demonstrating the distribution of domains across α and β chains and the location of α-β disulfides and α-α dimer disulfide bonds. [Adapted from Clemmons DR (2001) IGF-I receptor-mediated signal transduction, in *Targets for Growth Hormone and IGF-I Action* (Bouillon R ed), pp 17–28, Bioscientifica Ltd., Bristol, UK. Copyright © 2001 Bioscientifica Ltd. Used with permission.]

Cell-surface IGF-IR levels are regulated by the relative expression of its gene (LeRoith et al., 1995; Werner et al., 1995). A number of factors seem to determine expression, depending on the cell-type (Du et al., 1999, 2001; Maile and Clemmons, 2003). In turn, those levels of receptor expression govern key cellular processes such as apoptosis. For instance, in vascular smooth-muscle cells, oxidative stress diminishes receptor levels through a mechanism involving enhanced association of p53 with the IGF-IR gene promoter (Kavurma et al., 2007). IGF-IR signals to multiple antiapoptotic pathways, and

its overexpression generally enhances cell survival. Moreover, IGF-IR seems necessary for malignant cell transformation in some models of carcinogenesis, such as the Ewing's family of tumors (Toretsky et al., 1997). In prostate cancer cells, IGF-IR activation leads to the initiation of downstream mTOR signaling regulating the expression of survivin (Vaira et al., 2007), a member of the inhibitor of apoptosis gene family and an important regulator of cell proliferation and viability (Ambrosini et al., 1997). By introducing the dominant-negative mutant 486/STOP IGF-IR into M12 prostate cancer cells expressing high levels of wild-type IGF-IR, Wu et al. (2003) enhanced apoptosis through actions mediated by p38 mitogen activated protein kinase (MAPK).

Recent studies reveal that the levels of cell-surface IGF-IR are also governed by regulatory events occurring at the surface of the plasma membrane. As with multiple other tyrosine kinase receptors, ligand-induced endocytosis serves an important function in signaling through the recruitment of several proteins, including the adaptor protein 2 (AP-2) complex, dynamin, endophilin, and clathrin (Mellman, 1996; Schmid et al., 1998). The requisite recognition determinates for complex assembly have been localized. They are contained in the EH domain of the N terminus of epidermal growth factor receptor (EGFR) pathway substrate, Eps15, a 100-amino acid signature that is repeated three times (Salcini et al., 1997). EPS15 has been linked to EGFR endocytosis (Benmerah et al., 1998). A family of four EH domain-containing proteins has been identified, termed EHD1-EHD4 (Mintz et al., 1999). These EHD domains are located in the C termini. Rotem-Yehudar et al. (2001) have implicated EHD1 in the endocytosis of IGF-IR, in association with SNAP29. In their study, the authors demonstrate that a complex containing clathrin, α-adaptin of AP-2, small nuclear RNA-activating protein, and EHD1 colocalize to the endocytic vesicles. Over-expression of EHD1 retards the phosphorylation of mitogen-activated protein kinase and Akt and dampens IGF-I-provoked signaling substantially in transfected Chinese hamster ovary cells (Rotem-Yehudar et al., 2001).

The immediate consequence of IGF-IR activation involves tyrosine autophosphorylation at several residues resulting from intrinsic tyrosine kinase activity in the β subunit (Kato et al., 1993). Phosphorylation of tyrosine residues 1131, 1135, and 1136 plays important roles in the canonical signaling attributed to the receptor (Grønborg et al., 1993; Kato et al., 1994). These protein modifications in turn create binding sites for multiple docking proteins (Craparo et al., 1995; Dey et al., 1996). Among these are the insulin receptor substrates (IRS)-1, -2, -3, and -4 and the Src homology and collagen domain protein p66 Shc (Fig. 3). IRS-1 contains 21 tyrosine residues and serves a prominent role by interacting with several Src homology-2 (SH-2)-containing proteins involved in downstream signaling. The phosphorylation of

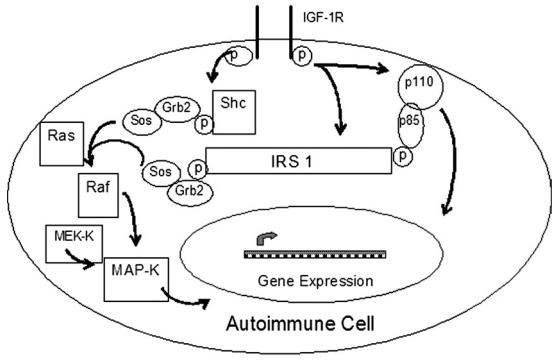

FIG. 3. Major components of IGF-R-linked signaling pathways. IRS-1 represents a central docking protein involved in the activation of MAP kinase and PI3 kinase pathways. Like IRS, Shc can be phosphorylated directly as a consequence of the receptor kinase. GF-R, growth factor receptor; MEK-K, MEK kinase; p, phosphorylation; Sos, Son of Sevenless. [Reprinted from Clemmons DR (2001) IGF-1 receptor-mediated signal transduction, in *Targets for Growth Hormone and IGF-1 Action* (Bouillon R ed), pp 17–28, Bioscientifica Ltd., Bristol, UK. Copyright © 2001 Bioscientifica Ltd. Used with permission.]

IRS-1 leads to phosphoinositol kinase 3/AKT activation and the adaptor protein Grb-2, which contains both SH-2 and SH-3 domains. Phosphoinositol kinase 3/AKT can associate with IRS-1, and the Grb-2 can bind the guanine nucleotide-releasing protein son-of-sevenless, which in turn participates in Ras activation (Egan et al., 1993), leading to phosphorylation of the serine/threonine kinase, Raf-1, and various components of the mitogen-activated protein (MAP) kinase pathway (Kecha et al., 2000). In addition, several phosphotyrosine phosphatases have been implicated in regulating IGF-I signaling. For instance, SH-2-containing phosphotyrosine phosphatase-2 (SHP-2) governs the duration of IGF-IR phosphorylation in smooth muscle cells (Maile and Clemmons, 2002a,b,c).

Multiple factors exert powerful regulatory influences on IGF-I-mediated signaling, insights that remain incompletely explored (Nagaoka et al., 1990; Lecka-Czernik et al., 2007; Martin and Baxter, 2007; O'Connor et al., 2008). Notable among them are abundant components of the extracellular matrix. When fibroblasts are cultured on fibronectin-coated culture surfaces, the abundance of IRS-1 increases (Lebrun et al., 2000), whereas a substratum rich in vitronectin facilitates its phosphorylation through interactions with focal adhesion kinase pp125 (Lebrun et al., 1998). Apparently, IGF-IR can associate with multiple integrins, and these interactions are cell-specific. The activation of $\alpha 3\beta 1$ by IGF-I in breast cancer cells can be up-regulated by plating them on a substratum enriched with thrombospondin (Chandrasekaran et al., 1999). The integrin receptor $\alpha V\beta 3$ regulates IGF-IR phosphorylation by influencing the rate of SHP-2 recruitment to the receptor complex (Maile and Clemmons, 2002a,b,c). A dynamic interplay exists between IGF-IR and the transmembrane proteins SHPS-1, a docking protein, and the $\alpha V\beta 3$ integrin (Clemmons and Maile, 2005). In lens epithe-

lium, the receptor coprecipitates with $\alpha 6$ (Walker et al., 2002), whereas in human chondrocytes, it associates with $\alpha 1\beta 1$ and $\alpha 5\beta 1$ (Shakibaei et al., 1999). It seems that $\alpha V\beta 3$ must be ligated to allow IGF-IR to fully influence vascular smooth muscle cell proliferation and migration. Blocking $\alpha V\beta 3$ with the monoclonal antibody LM609 attenuates IGF-I-dependent cell migration. A critical component to this signaling concerns the recruitment of SHP-2. SHP-2 is subsequently transferred to SHPS-1. This reaction requires that the latter become tyrosine-phosphorylated through an IGF-IR-dependent event (Pollak et al., 2004). Blocking the interaction between ligands and $\alpha V\beta 3$ enhances SHP-2 binding to IGF-IR, causing dephosphorylation of the receptor's tyrosine residues and dampening the signaling mediated through MAP kinase and phosphatidylinositol 3 kinase pathways (Maile and Clemmons, 2002a,b,c). The phosphorylation of tyrosines 785 and 773 on $\beta 3$ seems critical to IGF-I-dependent MAP kinase signaling and cell proliferation (Ling et al., 2003). Conversely, IGF-I activity enhances the avidity with which $\alpha V\beta 3$ binds ligands without altering maximum receptor binding capacity (Jones et al., 1996).

Another regulatory phosphatase, protein tyrosine phosphatase 1B, can also reduce the levels of IGF-IR phosphorylation (Buckley et al., 2002). Unlike the closely related EGFR and platelet-derived growth factor receptor, IR and IGF-IR fail to bind SH-2-domain-containing proteins but instead drive the phosphorylation of IRS and Shc proteins (White, 1997). Moreover, a number of potentially seminal findings have suggested that a cooperative relationship between IGF-IR and EGFR in signaling patterns exists and may prove to be cell type-specific (Roudabush et al., 2000). These receptors, separately and in aggregate, may form "clearing houses" for converging signals derived from a wide array of cross-talking pathways, including those involved in the actions of multiple hormones, cytokines, growth factors, agents of cell stress, and oxidative events (Rosen and Greenberg, 1996; Rosette and Karin, 1996; Moro et al., 1998; Carpenter, 1999; Hackel et al., 1999; Luttrell et al., 1999). In particular, IGF-I can promote Erk phosphorylation through the intermediate activation of Shc. This series of events might require IGF-IR dependent EGFR trans-activation (Roudabush et al., 2000). It is noteworthy that El-Shewy et al. (2004) have recently proposed a model in which the generation of EGFR ligands provoked by IGF-IR activation results in the trans-activation signaling of EGFR. This, in turn, leads to cell type-specific downstream signaling events. By transfecting an expression plasmid encoding heparin binding-epidermal growth factor-like growth factor/influenza virus hemagglutinin/Myc into human embryonic kidney 293 cells, they demonstrated that IGF-I elicited the rapid proteolysis of the fusion protein. Furthermore, IGF-I, EGF, and heparin binding-EGF-like growth factor could all enhance Tyr-1068 phosphorylation of endogenous EGFR and mimic EGF in driving EGFR internalization from the cell surface (El-Shewy et al., 2004). The paracrine nature of this relationship was established by demonstrating that IGF-IR$^+$ cells could respond to stimulation as did IGF-IR$^-$ cells in coculture. Responses of these receptor-null cells were abolished by inhibiting matrix metalloproteinases and EGFR activation.

IGF-IR may also serve as a substrate for γ-secretase. McElroy et al. (2007) have demonstrated that a 52-kDa C-terminal fragment of IGF-IR is generated both constitutively and at an increased level after treatment of mouse embryo fibroblasts with phorbol 12-myristate 13-acetate. Generation of the fragment, an appropriate substrate for γ-secretase, is presumed to be proceeded by the shedding of IGF-IR from the membrane surface. This would be mediated by one or more metalloproteinases, such as those belonging to the disintegrin and metalloproteinase domain-containing protein (ADAMs) family. The authors tested whether the putative C-terminal fragment of IGF-IR was indeed a γ-secretase substrate by treating cells with compound E, a specific inhibitor of the enzyme. The compound enhanced accumulation of the 52-kDa protein but limited production of the expected 50-kDa intracellular domain fragment that should result from γ-secretase cleavage activity (McElroy et al., 2007). In aggregate, based on these studies, IGF-IR signaling activity and abundance at the cell- surface seems to be regulated through a number of mechanisms involving interactions with a diverse array of molecules. These would include proteases involved in protein cleavage and recruitment of docking proteins.

C. Insulin-Like Growth Factor-I Binding Proteins

In addition to the relative concentrations of IGFs and the cell-surface density of IGF-IR, the abundance and profile of IGFBPs serve as important determinants of signaling by influencing IGF availability for binding to the receptor. The IGFBP family comprises six proteins exhibiting relatively high affinity for IGFs. In fact, their affinities for IGF-I generally exceed that of IGF-IR. They are synthesized by many tissues and cell types, and their relative levels are under hormonal control. For instance, sex steroids regulate IGFBP synthesis in breast, granulosa cells, and cultured osteoblasts (Mondschein et al., 1990). In addition to their relative levels determining the biological impact of IGF-I, IGFBPs can undergo post-translational processing, such as phosphorylation, glycosylation, and ubiquitination. Each of these protein modifications can profoundly alter IGFBP binding activity. Besides their functions as IGF-I carrier proteins, IGFPBs exert actions on target cells, either as ligated or unligated molecules (Hwa et al., 1999). Notable among them is IGFBP3, which has been implicated in the pathogenesis of several forms of cancer, including that of the prostate (Pollak et al., 2004). It is by virtue of the wide array of proteases associated with prostate cancer that IGFBP3 is degraded, freeing IGF-I in the

process. Specific proteases have been identified for each IGFBP. Among these are cathepsin, various matrix metalloproteinases, stromelysins, and kallikreins (Blat et al., 1994; Mañes et al., 1997, 1999).

IGFBPs generally serve to modulate growth factor activity, in large part by sequestering IGF-I and therefore determining the fraction that is available to act on target cells (Rosenfeld et al., 2000). Most of these circulate as approximately 150-kDa complexes containing an acid-labile subunit, IGFBP, and IGF-I. After the dissociation of this three-component aggregate, IGF-I/IGFBP comes out of circulation and crosses the endothelium to associate with target cell surface IGF-IR. With regard to its abundance and contribution to IGF-I carrying capacity, IGFBP3 is the most important (Firth and Baxter, 2002). IGFBPs can also enhance IGF-I activity, perhaps by facilitating its delivery to target cells (Wetterau et al., 1999). As a complex, IGF-I survival in the circulation is prolonged, its interactions with IGF-IR are modulated, and the IGFBPs might help target specific cells for IGF-I action. Moreover, IGFBPs help create concentration gradients for IGF-I and therefore determine the factor's impact on microenvironments surrounding target cells.

While a number of the biological effects ascribed to IGFBPs are dependent upon their association with IGF-I or IGF-II (Jones and Clemmons, 1995; Rajaram et al., 1997), increasing interest has driven further investigation into those actions that are IGF-I-independent. In addition to their roles as chaperones for IGF-I, at least some of the IGFBPs seem to act in the absence of bound IGF-I (i.e., in an unligated state). They bind multiple extracellular matrix components (Firth and Baxter, 2002). For instance, IGFBP1 contains an Arg-Gly-Asp (RGD) integrin recognition site situated in the carboxyl terminus (Drop et al., 1992) through which it can bind to the fibronectin receptor $\alpha5\beta1$ (Jones et al., 1993). IGFBP2 possesses a similar motif. In addition, a basic heparin-binding sequence in the thyroglobulin-like domains resembles those found in IGFBP-3, IGFBP-5, and IGFBP-6. IGFBP-3 interacts with the glycosaminoglycan moiety of proteoglycans (Baxter and Firth, 1995; Fowlkes and Serra, 1996). Similar domains on other IGFBPs allow analogous interactions on cell surfaces and within the extracellular matrix (Parker et al., 1996, 1998), although little insight currently exists concerning their biological function. One potential consequence of this basic heparin-binding domain concerns modulating the degradation of another related protein, IGFBP-4, the effects of which seem to be opposed by IGF-I (Verschure et al., 1996). It is possible that the basic region of IGFBPs inhibits protease activity (Fowlkes et al., 1997).

IGFBP3-provoked signaling seems to use retinoic acid receptor X, a nuclear transcription factor, to which it binds with relatively high affinity (Lee and Cohen, 2002). This binding protein complexes with RXR-α within the cell nucleus (Liu et al., 2000). Moreover, IGFBP3-induced apoptosis is abolished in cells in which RXR-α is knocked out. Thus, the aggregate of RXR and IGFBP3 proteins conveys functional importance. In addition, specific membrane binding of IGFBP3 has been demonstrated in chick embryonic cells (Delbé et al., 1991), Hs578T (Oh et al., 1993) and MCF-7 breast cancer cells (Ricort et al., 2002). The type V TGF-β receptor can bind IGFBP3 (Leal et al., 1999). A putative 420-kDa membrane receptor for IGFBP5 that exhibits serine kinase activity has been demonstrated on osteoblasts (Andress, 1995; Berfield et al., 2000). IGFBP5 can also associate with TS-1, which in turn attenuates the integrin associated protein-TS-1 complex and therefore diminishes IGF-IR phosphorylation and activity (Moralez et al., 2005). IGFBP3 directly induces phosphotyrosine phosphatase activity in MCF-7 cells (Ricort and Binoux, 2002). Thus, it is possible that this protein exerts its modulating influence on IGF-I signaling by dampening tyrosine phosphorylation (Ricort and Binoux, 2001).

III. Emerging Insights into Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-I Receptor: Roles in Immune Integration

Although endocrine function is intimately intertwined with growth and development, the potential relationship between immune function and growth factors such as IGF-I has remained poorly characterized until relatively recently. With the growing realization that diverse regulatory pathways often converge, a number of studies have demonstrated the importance of GH, IGF-I, and IGF-IR to many aspects of immune function. In addition, immune reactions and the inflammation with which they are often linked have been shown to affect normal growth and patterns of tissue remodeling occurring in wound repair. Although these interactions could have been predicted from the well known deleterious effects of chronic inflammatory disease on child growth and development, we now have gained critical insights into their mechanistic basis. Linking these biological functions is the complex interplay between cytokines and growth factors, including IGF-I. This topic has been reviewed recently (O'Connor et al., 2008). In brief, proinflammatory cytokines seem to dampen several components of the IGF-I pathway. Many of the cytokines share common signaling components, such as the Erk 1/2 MAP kinase. Thus, molecular events initiating signaling down a common pathway can modify the availability and activities of shared postreceptor docking proteins and thereby influence the magnitude and quality of cellular responses emanating from receptor/ligand interactions. As an example, levels of IGF-I trend downward as a consequence of aging and chronic disease (Moldawer and Copeland, 1997; Grounds, 2002). Thus, if all other factors remain constant, the influence exerted by the endogenous IGF-I/IGF-IR pathway on immune function and inflammation mediated through the signaling pathways shared with IGF-IR might diminish as an individ-

ual ages or becomes chronically ill. Conversely, children diagnosed with one of several diseases associated with chronic illness exhibit alterations in growth and development attributable to GH/IGF-I dysfunction.

A. Hematopoiesis

Soon et al. (1999) reported findings from studies examining how IGF-IR and IL-4R signaling interact in 32D myeloid precursor cells. These IL-3-dependent cells express IR but lack both IRS-1 and IRS-2. IL-4 fails to elicit any response in 32D cells. However, when either IRS-1 or IRS-2 is coexpressed with IL-4R, the cells respond to IL-4 robustly (Wang et al., 1993). When IGF-IR is overexpressed in 32D cells, the activated receptor initiates DNA synthesis. It is noteworthy that IGF-IR transfectants were also responsive to IL-4 in the absence of either IRS-1 or IRS-2 (Soon et al., 1999). The effects of IGF-I and IL-4 seemed synergistic and were unrelated to changes in PI3 kinase activity, but the Src-homology-collagen/Grb2/MAPK pathway played a critical role (Soon et al., 1999). Moreover, c-*myc* gene up-regulation and enhanced Erk2 and STAT6 activities were positively correlated with the effect of IL-4 on cell proliferation in these IGF-IR transfectants. hIGF-I promotes hematopoietic growth in vivo in mice (Tsarfaty et al., 1994). In mouse syngeneic bone marrow transplant models, GH and IGF-I administration enhances reconstitution of the immune system (Murphy et al., 1992a,b,c; de Mello-Coelho et al., 1997). Recombinant GH also enhances hematopoietic reconstitution after syngeneic bone marrow transplantation in mice (Tian et al., 1998). Using three different but extensively characterized mouse models of allogeneic bone marrow transplantation, Alpdogan et al. (2003) demonstrated that $CD4^-CD8^-CD3^-Thy^-1.2^+$ lymphoid and myeloid reconstitution after marrow transplantation was enhanced by IGF-I. In that study, thymic precursor cell populations were expanded by IGF-I administration (Alpdogan et al., 2003). The $CD25^+CD44^+CD4^-CD8^-CD3^-Thy^-1.2^+$ and $CD25^+CD44^-CD4^-CD8^-CD3^-Thy^-1.2^+$ subsets were increased. On the other hand, overall thymic cellularity was unaffected. IGF-I increased the frequency of splenic myeloid cells and pro-B, pre-B, and mature B cells in allogeneic bone marrow transplant recipients. Donor-derived peripheral splenic $CD3^+$T cells became more abundant and exhibited enhanced proliferative responses to mitogens. Moreover, the effects of IL-7 on B cells were substantially promoted by IGF-I but not those actions exerted on T cells. The study also examined the impact of IGF-I on the development of graft-versus-host reactions in both MHC matched and mismatched hosts and found no effects on either morbidity or mortality among the animals (Alpdogan et al., 2003).

B. Thymus Development and Function

Regulation of thymic development and physiologic function involves numerous intersecting molecular signals. Although it was thought to function only in childhood, much evidence now supports the concept that thymic activity persists well into adulthood (Poulin et al., 1999). Moreover, certain pathological states seem to promote increased activity within the thymus (Choyke et al., 1987; McCune et al., 1998). Among the supporting factors, IGF-I and GH target thymic epithelial cells, where they synergistically promote the action of anti-CD3 in stimulating proliferation (Savino et al., 2002). Kooijman et al. (1995a,b,c) reported that human thymocytes display 257 ± 28 IGF-I binding sites/cell with a K_d of 0.12 ± 0.01 nM. Precursor thymocytes represent pluripotential $CD3^-CD4^-CD8^-$ cells that differentiate into mature $CD3^+CD4^+CD8^-$ or $CD3^+CD4^-CD8^+$ phenotypes. Mature thymocytes express IGF-IR. Immature cells ($CD4^-CD8^-$) display 3- to 4-fold higher receptor levels than do immature $CD3^{-/low}CD4^+CD8^+$ cells and mature $CD4^+CD8^-$ or $CD4^-CD8^+$ cells. IGF-I directly stimulates DNA synthesis in human thymocytes (Kooijman et al., 1995a,b,c). Besides thymocytes, GH and IGF-I have been shown to regulate nonlymphoid components of the thymus. Thymic epithelial cells from human and murine sources express IGF-IR mRNA, as do established cell lines (de Mello-Coelho et al., 1997). In culture, IGF-I induces the synthesis of the zinc-binding nanopeptide serum thymic factor (i.e., thymulin) in human thymic epithelial cells (Timsit et al., 1992; de Mello-Coelho et al., 1997). The authors of these studies found that the plasma levels of thymulin were increased significantly in 21 patients with acromegaly compared with 30 control subjects (Timsit et al., 1992). The effects of IGF-I on the thymic cell population are time-dependent and seem specific. Moreover, the cross-talk between the thymic epithelium and lymphoid cells can be modulated by GH through IGF-I (de Mello-Coelho et al., 1997). IGF-I and IGF-IR mRNA can be detected in murine and human thymic epithelial cells (de Mello Coelho et al., 2002). GH enhances IGF-I synthesis in murine and human thymic epithelial cells. Moreover, treatment of these cells with IGF-I results in increased $CD4^-CD8^-CD90^+$ (Thy-1^+) T cell adhesion, suggesting the possibility that the growth factor might indirectly influence intrathymic T cell differentiation and migration (de Mello Coelho et al., 2002). Atrophy of the thymus is a recognized consequence of substantial insulin deficits in diabetes mellitus. The thymic atrophy found in animal models of insulin-deficient diabetes can be reversed with exogenous IGF-I at doses insufficient for restoring glycemic control (Binz et al., 1990). That result suggests the possibility that IGF-I, and perhaps insulin, might exert actions on the thymus that are independent of their impact on blood glucose levels. Both IGF-I and IGF-II can increase the number of $CD4^+CD8^+$ immature T cells in rat thymus and spleen (Hinton et al., 1998). They also enhance repopulation of atrophic thymus after cyclosporine treatment (Beschorner et al., 1991). Although IGF-I failed to prevent glucocorticoid-induced thymocyte apoptosis in rats, it reduced the death rate among lymphocytes in the spleen

and protected modestly splenic B and T cell loss (Hinton et al., 1998). It also enhanced the recovery of $CD4^+CD8^+$ immature intrathymic T cells and depressed the abundance of $CD8^+$ cells (Hinton et al., 1998). When administered to 9-month-old male mice, both splenic and thymic weights increased, and these tissues contained substantially increased numbers of $CD4^+$ T cells (Clark et al., 1993). In addition, splenic B cells were also more numerous in these animals. GH helps reverse age-related decline of thymopoiesis in animal models (Clark et al., 1993; Kelley et al., 1996; Clark, 1997). In adult patients infected with HIV-1, GH increased thymic mass and the abundance of circulating $CD4^+$ T cells (Napolitano et al., 2002). In a considerably more comprehensive prospective clinical trial, these same investigators reexamined GH effects in adults infected with HIV-1 (Napolitano et al., 2008). They concluded that the thymic output was increased because the frequency of circulating naive and total T cells was elevated, as was T cell receptor rearrangement excision circles (Napolitano et al., 2008). Those findings imply that GH might reverse thymic involution in immunodeficient human subjects and therefore its administration might constitute therapy. Although the administration of hGH and rIGF-I in patients infected with HIV failed to influence significantly the abundance of $CD4^+$ T cells, alter the profile of RA and RO $CD4^+$ subsets, or affect several other immunologic endpoints, the authors of a pilot study concluded that these agents could modestly improve HIV-specific immune function (Nguyen et al., 1998). These observations could carry broader implications concerning a strategy for preserving immune function in the context of various disease processes such as retroviral illness and the decline resulting from normal aging.

C. Immunocoordination

Besides their impact on discrete components of the immune system, IGF-I and IGF-II seem to modify several aspects of inflammation, at least in part by influencing the actions of cytokines and other small molecule mediators. These same mediators can in turn alter the abundance of IGF-I and modulate its actions on target tissues. Thus various components of the inflammatory machinery and the IGF-I pathway share a complex relationship that manifests in several ways. As an example, IGF-I can increase survival in rats treated with D-galactosamine and LPS, a strategy used to induce experimental acute hepatic failure (Hijikawa et al., 2008). When administered before the D-galactosamine and LPS, IGF-I prevented the biochemical stigmata of liver failure, such as elevations in bilirubin and transaminases. Upon histologic examination, the growth factor seemed to decrease hepatic apoptosis and neutrophil infiltration. This effect seems to result from IGF-I blocking the elevation of IL-1β, TNF-α, and neutrophil chemoattractant 1 associated with D-galactosamine and LPS administration. IGF-I additionally reduces the production of nitrous oxide by inhibiting the inductive effects of LPS and D-galactosamine on nitric-oxide synthase mRNA and protein levels in the liver. These effects seem to be independent of nuclear factor-κB (Hijikawa et al., 2008). When TNF-α signaling in skeletal muscle is activated through the c-Jun N-terminal kinase, the signal activity provoked by IGF-I can be attenuated through changes induced in the conformation of IRS-1 and its phosphorylation (Grounds et al., 2008). In children with extensive thermal burns, the administration of IGF-I in combination with IGFBP3 as a continuous infusion at a rate of 1 to 4 mg/kg/day, reduced serum levels of IL-1β, TNF-α, C-reactive protein (CRP), α_1-acid glycoprotein, and complement C-3 (Jeschke et al., 2000). In contrast, the serum levels of retinol-binding proteins, prealbumin, and transferrin were increased by the infusion. The inflammatory status of patients with peripheral arterial disease affects serum levels of IGF-I and IGFBP-3 (Brevetti et al., 2008). In patients manifesting either ulcerative colitis or Crohn's disease, circulating levels of IGF-I are inversely correlated with erythrocyte sedimentation rate and C-reactive protein (Street et al., 2003). On the other hand, IGFBP-2 levels are positively correlated with erythrocyte sedimentation rate and IL-1β levels. In black male smokers, IGF-I levels are inversely correlated with those of C-reactive protein (Colangelo et al., 2009).

IV. Impact of Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-I Receptor on Immune Cell Lineages

A. T Lymphocytes

T cells display many different surface growth factor receptors, including IGF-IR (Schillaci et al., 1998). IGF-I, IGF-II, and insulin bind to the surface of T and B cells. High-affinity binding sites for insulin ($K_d = 2 \times 10^{-10}$ M) were found by Helderman and Strom (1978) on activated B and T cells, and IR was proposed by the authors to represent a marker of lymphocyte activation. Lee et al. (1986) were among the first to demonstrate IGF-IR expression by some, but not all, T cell lines derived from patients with lymphoid malignancies. High-affinity, saturable binding of ^{125}I-IGF-I was subsequently demonstrated on both activated and resting human T cells with a K_d of 1.2×10^{-10} M (Tapson et al., 1988). The number of binding sites increases from approximately 45 high-affinity sites per resting cell to 330 sites per activated cell. Moreover, those studies demonstrated that IGF-I can induce T-cell proliferation and chemotaxis (Tapson et al., 1988). The binding capacity for IGF-I is greater on rat $CD4^+$ compared with $CD8^+$ T cells. Moreover, binding sites on $CD8^+$ T cells seem to exhibit a relatively lower affinity for the ligand than those on their $CD4^+$ counterparts (Xu et al., 1995). IGF-IR levels displayed by rat lymphocytes increase af-

ter activation with concanavalin A in both $CD4^+$ and $CD8^+$ T cell subsets.

The consequences of IGF-IR expression to lymphocyte function have been examined in several different studies employing a wide array of experimental models and technical approaches. Yang et al. (2002) studied human cord blood lymphocytes and the impact of knocking down IGF-IR expression with antisense oligonucleotides on cell function. They found that receptor abundance was increased by cell activation with either phytohemagglutinin or pokeweed mitogen and that reducing IGF-IR levels resulted in depressed cell proliferation and reduced IgM production and cytokine expression (Yang et al., 2002). Their studies apparently were conducted in culture medium supplemented with 10% fetal calf serum but otherwise in the absence of IGF-I. T-cell generation in neonates focuses on maintaining a preset optimal clonal size and is not necessarily geared toward attaining maximal repertoire diversity (Schönland et al., 2003). Thus, naive T cells remain capable of cellular replication, even late in life. A host of factors allowing expansion supports attainment of the preset post-thymic T cell clonal size. Among them, IL-7 plays an important role, especially during neonatal life, but may also exert an influence during adulthood (Schönland et al., 2003). Whether IGF-I and its signaling pathways also play some role in determining how T-cell compartments are filled throughout life remains an open question. However, given the importance of IL-7 in that process and how IGF-I potentiates the actions of IL-7 in pro-B cell expansion (Gibson et al., 1993), a similar influence on T cells seems likely.

Both IGF-I and IGF-II play important roles in the development and function of T cells. IGF-I can activate T cell Akt and thereby enhance lymphocyte survival (Walsh et al., 2002). However, both IGF-I and insulin can also suppress immune responses. In C57BL/6 mouse splenocytes, IGF-I profoundly blocked IL-2-dependent cell growth (Hunt and Eardley, 1986). Although not as potent, insulin had similar effects. These were evident in both T and B cell-enriched splenocyte preparations treated with IL-2 and were also observed in unfractionated splenocytes treated with Concanavalin A and LPS. The EL4 thymoma cell line exhibited growth retardation in response to IGF-I similar to that observed in primary splenocytes, where the growth factor was 100 to 1000 times more potent than insulin. Proliferation of the mouse T cell line CTLL2 cells is dependent on IL-2 but is inhibited by IGF-I. These cells were less sensitive to the inhibitory actions of IGF-I than were splenocytes. With regard to responses in vitro, in a plaque-forming cell assay, IGF-I and insulin suppressed antibody production (Hunt and Eardley, 1986). The suppressive effects of IGF-I on IL-2-dependent cell proliferation could not be overcome with increasing concentrations of the latter agent. The suppressive effects were time-dependent and evolved over many hours of incubation. Thus, IGF-I and insulin, although generally enhancing lymphocyte proliferation (Heulin et al., 1982; Schimpff et al., 1983; Walsh et al., 2002), can also block IL-2-dependent lymphocyte growth and function.

Thymic IGF-I, IGF-II, and IGF-IR mRNAs are expressed as early as fetal day 14 in mice (Kecha et al., 2000). The IGF-II transcript declined in the postnatal period, but a weak reverse transcription-polymerase chain reaction signal remained detectable at week 7 (Kecha et al., 2000). In that report, culture-based studies of fetal thymic organ suggested that interruption of the IGF-I and IGF-II pathways results in potentially important and divergent effects on the differentiation of double-negative $CD4^-CD8^-$ cells compared with $CD4^+CD8^+$ cells and after their progression to mature $CD4^+CD8^-$ and $CD4^-CD8^+$ phenotypes (Kecha et al., 2000). When administered to animals, IGF-I expands the T-cell population (Clark et al., 1993). IGF-II also enhances T-cell development, as was strongly suggested in studies using transgenic mice overexpressing that protein where human IGF-II production was under the control of the $H2K^b$ gene promoter (Kooijman et al., 1995a,b,c). Transgenic thymus in 1-week-old mice from the two different lines contained 36 and 68% more thymocytes than did control animals (Kooijman et al., 1995a,b,c). At 4 weeks of age, IGF-II expression resulted in enhanced thymic cellularity. This increase could be accounted for by the emergence of more numerous early $CD4^-CD8^-$ and $CD4^-CD8^{dim}$ cells, intermediate $CD4^+CD8^+$ cells, and mature thymocytes with the $CD3^+CD4^+CD8^-$ or $CD3^{++}CD4^-CD8^+$ phenotypes. As the mice continued to age, differences between thymocyte populations in transgenic and control animals disappeared.

Stage of maturation determines the level of IGF-IR displayed by developing T cells, as assessed by recognition with the specific monoclonal antibody αIR3. Receptor density was quantified on human peripheral T cells (Kooijman et al., 1995a,b,c). Eighty-seven percent of $CD4^+CD45RA^+$ cells and 66% of $CD8^+CD45RA^+$ cells stained for the receptor (Kooijman et al., 1995a,b,c). In contrast, 37 and 38% of the $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ memory T cells, respectively, exhibited the $IGF\text{-}IR^+$ phenotype (Kooijman et al., 1995a,b,c). T-cell expression of IGF-IR undergoes down-regulation as differentiation proceeds. Thus double-negative $CD4^-CD8^-$ cells exhibit 3- to 4-fold higher levels of surface receptor display than do either double-positive or single-positive cells. IGF-IR levels were markedly lower in activated T cells, both in vitro and in vivo. $CD4^+CD45RO^+$ cells activated by phytohemagglutinin or after exposure to recall antigens in vitro display considerably lower levels of IGF-IR than do inactive lymphocytes (Kooijman et al., 1995a,b,c; Schillaci et al., 1998; Segretin et al., 2003). This reduction may be transient and associated with depressed steady-state IGF-IR mRNA levels (Segretin et al., 2003). A similar finding could be demonstrated in Jurkat cells, and addition of IGF-I to the culture me-

dium enhanced this effect (Schillaci et al., 1998). These findings contrast with those reported by Tapson et al. (1988) and may reflect differences in experimental conditions or how cell maturation studies were performed. In addition, monocyte-depleted peripheral human T cells activated with immobilized anti-CD3 were found to display up-regulated IGF-IR, IGF-IIR, and IR (Johnson et al., 1992). Those cells proliferated in response to both IGF-I and IGF-II, effects that could be blocked with the IGF-IR-blocking antibody αIR3. IGF-IR expression can also be up-regulated in T cells through CD28 receptor cross-linking or by activating the CD80/CD86 pathway (Walsh and O'Connor, 2000). Moreover, blocking IGF-IR on TCR- and CD28-engaged T cells decreases lymphocyte survival in the presence of IL-2 (Walsh and O'Connor, 2000). CD28 activation enhances IGF-IR display on Jurkat cells and treatment with IGF-I conveys resistance to Fas-mediated apoptosis (Walsh and O'Connor, 2000). IGF-I enhances the maturation of T cells collected from cord blood (Tu et al., 2000) and blocks spontaneous apoptosis and the programmed cell death induced by phytohemagglutinin. It down-regulates interferon γ R2 chain display on the surface of human T cells (Bernabei et al., 2003), resulting in desensitization of these cells to interferon γ-dependent STAT-1 signaling. It also activates Akt (aka PKB) and c-Jun N-terminal kinases, resulting in resistance to Fas-mediated apoptosis (Walsh et al., 2002). On the other hand, IGF-I elicits the production of IL-10 in human T cells through a modest increase in IL-10 mRNA (Kooijman and Coppens, 2004). In cultured 32D hematopoietic cells overexpressing IGF-IR, treatment with IGF-I and IL-4 enhanced DNA synthesis in the absence of IRS expression (Soon et al., 1999). The SH-2/Grb2/MAPK pathway seems crucial to the mitogenic effects of both agents.

Activation of the signaling pathways downstream from IGF-IR and IR increases lymphocyte metabolism. The overall capacity for increased energy turnover accompanying T-cell activation is regulated by insulin (Frauwirth and Thompson, 2004). Two signals provoke cell activation: primary signals are directed through the TCR/CD3 complex, and secondary signals are provided by activated costimulatory receptors such as CD28 (Parry et al., 1997; Frauwirth et al., 2002). Enhanced glucose uptake is mediated through the transcriptional up-regulation of the Glut1 transporter accompanied by the translocation of Glut4 transporter to the cell surface (Barthel et al., 1999; Wang et al., 1999). Moreover there is an overall shift to aerobic glycolysis (Buttgereit et al., 2000). Oxygen demand after activation occurs rapidly but is overshadowed by a shift to glycolysis. A major consequence of the metabolic profile associated with cell activation is accelerated lactate generation, similar to that observed in tumor cells. The signaling downstream from both IR and IGF-IR is mediated through Akt/PKB (Barthel et al., 1999). CD28 activation, like that of IR and IGF-IR, culminates in the recruitment of Akt to the cell surface by phosphatidyl inositol triphosphate, where it becomes phosphorylated through the actions of PI3 kinase-responsive kinase PDK-1.

Aging rodents exhibit diminished responsiveness to pathogens. This shift is associated with reduced cellularity and frank involution of the thymus (Hadden et al., 1992; Miller, 1996; Montecino-Rodriguez and Dorshkind, 1997; Tian et al., 1998). A potential strategy for reversing these senile changes in thymic vitality involves administration of either GH or IGF-I. These agents have been examined for their potential to expand T-cell populations in animals. Eighteen-month-old mice were administered IGF-I, bone marrow cells from younger mice, or a combination of the two (Montecino-Rodriguez et al., 1998). Cellularity of the thymus in animals receiving both was enhanced considerably more than in those receiving only one of these treatments. This report contained studies conducted in vitro, demonstrating that IGF-I might potentiate thymic colonization by T cells derived from the bone marrow (Montecino-Rodriguez et al., 1998). Thus, alteration of the hematopoietic as well as endocrine defects associated with normal aging might reverse thymic involution.

DW/J dwarf mice produce less prolactin and GH than is found in control mice (Duquesnoy and Pedersen, 1981). They also manifest abnormalities in T-cell development (Fabris et al., 1971), including deficits in the abundance of $CD4^+CD8^+$ thymic cells (Murphy et al., 1992a,b,c). Treatment of these animals with GH could restore the deficient T-cell progenitor pool within the thymus, enlarge this gland, and enhance peripheral T-cell function. These authors explored the impact of prolactin treatment in the same DW/J mice and reported that its effects were markedly distinct from those of GH (Murphy et al., 1993). Prolactin decreased further thymic cellularity but enhanced both the frequency and activity of peripheral T lymphocytes in heterozygous and dwarf mice. In contrast, GH administration had little influence on antigen-specific responses (Murphy et al., 1993). GH did enhance human T-cell engraftment in SCID mice (Murphy et al., 1992a,b,c). This was related to increased resting and anti-CD3-activated T cell adhesion to intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and fibronectin (Taub et al., 1994). Moreover, analysis of the mechanisms involved revealed that β1 integrin mediates binding to vascular cell adhesion molecule-1 and fibronectin, whereas β2 is necessary for adhesion to intercellular adhesion molecule-1. Furthermore, the impact of GH on increasing human T cell engraftment could be blocked following preincubation with anti-β1 and anti-β2 antibodies (Taub et al., 1994). The hormone could also up-regulate the random migration of resting and activated human T cells, but the effects were substantially less pronounced than those elicited by regulated upon activation, normal T-cell expressed (RANTES),

even when the concentration of RANTES was 100-fold lower.

Like many other regulatory molecules, states of resistance to the actions of IGF-I have been described, usually in the context of growth abnormalities (Jain et al., 1998). In a series of studies, Geffner et al. (1993, 1995) established a number of T and B cell lines derived from adult Efe Pygmies or from Lese farmers who are their neighbors in Zaire. The basis for the growth abnormalities found in Efe pigmies concerns a substantially diminished compliment of IGF-IR molecules on the cell-surface and an absence of receptor autophosphorylation in response to physiological concentrations of IGF-I (Hattori et al., 1996). The stature attained by these Lese farmers is intermediate between those of urban black Africans and Pygmies. Interbreeding between these populations is known to occur. The investigators performed clonal proliferation assays in which T cells from the two populations and a third control cohort from North America were incubated with GH, IGF-I, insulin, or appropriate controls and cultures were enumerated after 4 to 7 days. T cells from the Efe Pygmies were found to be completely resistant to the actions of IGF-I. Lese farmer-derived clones exhibited intermediate responses. On the other hand, responses to insulin were indistinguishable among the three T cell donor sources, even when the concentrations used were extremely high. The absence of attenuation in the clones from Pygmies was surprising to the authors because they had demonstrated that responses to high concentrations of insulin are usually mediated through the promiscuous activation of IGF-IR (Geffner et al., 1987, 1992). However, in the case of the Pygmy-derived clones, IGF-IR apparently was uninvolved in the insulin response (Geffner et al., 1993). Similar studies conducted in B cells from these same Efe Pygmy donors revealed similar results: IGF-I failed to elicit responses, even at extremely high concentrations (Cortez et al., 1996), despite the comparable responses in cells from all three sources to phorbol 12-myristate 13-acetate.

B. B Lymphocytes

B cells play diverse roles in immune function by virtue of their further differentiation into immunoglobulin-secreting plasma cells, generation of cytokines, and their importance in antigen presentation. A critical component of B-cell development concerns how committed precursor cells from the hematopoietic lineage undergo immunoglobulin heavy chain gene rearrangement (Kincade et al., 1989). This leads to the expression of light chains and ultimately to the release of mature B cells from the bone marrow. A number of exogenous factors emanating from marrow stromal cells provide molecular support for early B-cell proliferation. Specifically, B-lineage progenitor cells in which Ig gene rearrangement has already been initiated, but before any detectable Ig light or heavy chains are produced, can undergo expansion in the presence of IL-7 when cocultured with stromal cells. Among these stromal factors, IGF-I drives B-cell differentiation (Landreth et al., 1992). In addition, IGF-I enhances IL-7-dependent B-cell proliferation in concert with c-*kit* ligand (Landreth et al., 1992) and potentiates IL-7 promotion of pro-B-cell expansion (Gibson et al., 1993). Cell lines derived from 14-day-old mouse fetal liver cells proliferate when cocultured with stromal cells cloned from S10 in the presence of IL-7. The proliferation of these lines could be enhanced with the addition of either c-*kit* ligand or IGF-I (Gibson et al., 1993). Moreover, the effects of c-*kit* ligand and IGF-I were additive, reflecting the distinct signaling pathways each used to activate these cells. Signaling components lying downstream from IGF-IR may condition B-cell function and responses to additional factors. IRS-1 overexpression in B lymphocytes derived from transgenic mice alters the density-dependent production of IgE and IgG1 in vitro and enhances IgE responses in those animals (Kelly-Welch et al., 2004). In contrast, IL-4-mediated proliferation and its impact on apoptosis were unaffected in mice harboring the IGF-IR transgene. When administered in vivo, IGF-I enhances the population of intrasplenic B cells through increased proliferation of mature cells (Clark et al., 1993; Jardieu et al., 1994). In bone marrow, the number of B cells increased after IGF-I administration in normal adult mice and those receiving lethal irradiation followed by reconstitution with syngeneic bone marrow (Jardieu et al., 1994). Studies conducted in vitro disclose that the differentiation of human $CD34^+$ bone marrow cells was substantially impeded by down-regulating the IGF-I pathway of MS-5 cells in coculture (Taguchi et al., 2006). These studies implicate IGFBP-6 as a necessary component of IGF-I-dependent B cell differentiation, whereas IGFBP-3 acted as an inhibitor of that process.

Plasma cells also respond to IGF-I. DNA synthesis increases substantially in four myeloma cell lines treated with IGF-I in vitro in the absence of IL-6 (Jelinek et al., 1997). In addition, both IGF-I and IGF-II enhance the proliferative action of IL-6 in three of these cell lines. The effects were absent in normal B cells, suggesting that the actions of IGF-I on B cells may diverge in malignant cells. Both IGF-IR and IR are expressed at higher levels by myeloma cell lines than those found in B lymphoblastoid lines (Freund et al., 1994). Plasma cell metabolism may also be targeted by IGF-I. In the RPMI 8226 line, cells express high-affinity binding sites for both insulin and IGF-I (Freund et al., 1993). IGF-I increases IGF-IR phosphorylation and PI3 kinase activation, enhances DNA synthesis, and up-regulates lactate production in these cells. In the IL-6Rα-expressing human myeloma cell line NOP2, IL-6 induces phosphorylation of IGF-IR, an action that cannot be blocked with Janus kinase 2 inhibitors but is duplicated in IL-6Rα-transfected U266 cells (Abroun et al., 2004). Moreover, IL-6Rα colocalizes with IGF-IR in lipid

rafts. The authors of the study also reported that IL-6 leads to the activation of STAT3, Erk1/2, and Akt/PKB, which they suggest is a consequence of IGF-IR phosphorylation. These findings identify a potential molecular basis for Janus kinase 2-independent IL-6 signaling mediated through IGF-IR. Moreover, they define a mechanism for cross-talk between two receptors that had been previously shown to independently support myeloma cells and their biosynthetic activities.

IGF-I can also influence antibody expression and class switching by plasma cells. For example, administration of IGF-I to mice results in elevated levels of antibodies (Robbins et al., 1994). Peripheral human B cells selectively bind IGF-I (Stuart et al., 1991). The binding is saturable and can be displaced with unlabeled IGF-I and by high concentrations of insulin. IGF-I binds to a 130-kDa protein in B cells. Human lymphoblasts synthesize IGFBP2 and IGFBP4 (Neely et al., 1991). Baudler et al. (2005) reconstituted Rag2-deficient C57BL/6 mice with fetal liver cells from IGF-IR(−/−) mice. T-cell-independent humoral responses to the type 2 antigen 4-hydroxy-3-nitrophenyl acetyl Ficoll were substantially diminished, whereas those against the T-cell-dependent antigen 4-hydroxy-3-nitrophenyl acetyl-chicken globulin were unaltered. B-cell development remained normal in IGF-IR-deficient chimeras, as did T-cell differentiation. In these animals, IGF-I enhanced immunoglobulin production, an effect that proved independent of B-cell proliferation (Baudler et al., 2005). Kimata and Yoshida (1994a,b) examined human B cells from individuals undergoing tonsillectomies for chronic tonsillitis. In addition, they analyzed several Epstein-Barr virus-transformed lymphoblastoid cell lines, including GM-1056, CESS, GM-1500, GM-3332, SKW, and CBL, and found that both GH and IGF-I enhanced Ig production in all of these cell types. It is noteworthy that the actions of GH were not dependent on an intermediate production of IGF-I (Kimata and Yoshida, 1994a,b). These same authors reported that GH and IGF-I induce IgG4 and IgE production in tonsillar B cells from control donors depleted of $sIgE^+$, treatments that left IgGM, IgG1, IgG2, IgG3, and IgA production unaffected (Kimata and Fujimoto, 1994). Unlike IGF-I, IGF-II and insulin failed to affect IgG4 or IgE synthesis. In these studies, too, the actions of GH seem to be unrelated to IGF-I production, because neutralizing antibodies directed against the growth factor failed to attenuate the impact of GH. It would seem, therefore, that IGF-I and GH may selectively induce IgG4 and IgE production through a class-switching mechanism (Kimata and Fujimoto, 1994). It is possible that GH signals through pathways that are independent of those involving the production of IGF-I. However, the absence of attenuation in studies in which anti-IGF-I antibodies are used to block GH actions does not entirely rule out a role for IGF-I. Interferons α and β could block the induction by IL-4 and IL-13 of IgG4 and IgE but not that of GH or IGF-I. Moreover, anti-CD40 antibodies failed to block the induction of Ig by either GH or IGF-I (Kimata and Fujimoto, 1994). Thus, it would seem that IGF-I and GH induce Ig class-switching in a manner that does not involve the IL-4 or IL-13 signaling pathways.

C. Monocyte-Macrophage Lineage

Human macrophages and granulocytes display IGF-IR. A number of IGF-I binding sites (1390 ± 467) were detected possessing a K_d of 2.3 ± 0.9 nM (Kooijman et al., 2002). IGF-I attenuated spontaneous apoptosis in these cells. Activated macrophages also express IGF-IR, whereas binding sites for ^{125}I-labeled IGF-I were absent on cells before activation (Rom and Pääkkö, 1991). This binding was quenched with excess unlabeled growth factor or with anti-IGF-IR monoclonal antibodies, attesting to its specificity. Alveolar macrophages, obtained from patients with idiopathic pulmonary fibrosis, produce a polypeptide growth factor that promotes fibroblast proliferation (Bitterman et al., 1983). This factor was subsequently identified as IGF-I (Rom et al., 1988). In that study, the 26-kDa macrophage-produced polypeptide was neutralized with an anti-IGF-I monoclonal antibody, could displace ^{125}I-IGF-I from IGF-IR on the surface of human lung fibroblasts, and provoked the phosphorylation of a tyrosine residue on an artificial IGF-IR substrate. In contrast, circulating monocytes fail to generate IGF-I, but tissue-infiltrating cells can be provoked to produce the growth factor by a number of agents (Rom et al., 1988; Kirstein et al., 1992). These act on macrophages through multiple signaling pathways. For instance, prostaglandin E_2 induces IGF-I expression through the generation of cAMP and the activation of protein kinase A (Fournier et al., 1995). Colony-stimulating factors also induce in macrophages the synthesis of IGF-I at a pretranslational level (Arkins et al., 1993), as does TNF-α (Fournier et al., 1995).

It seems that in idiopathic pulmonary fibrosis, a disease associated with increased fibroblast proliferation, IGF-I levels correlate well with disease severity. The production of IGF-I by macrophages in this disease may determine, at least in part, disease severity (Uh et al., 1998). This is controlled by several factors, including hyaluronan, acting through the surface receptor, CD44 (Noble et al., 1993). Induction of IGF-I by glycosaminoglycan was found to be mediated through an intermediate up-regulation of TNF-α, an action that was enhanced by IL-1β. On the other hand, interferon γ attenuates IGF-I synthesis in rat macrophages by lowering levels of its steady-state mRNA. These actions are mediated at the level of IGF-I gene transcription rather than as a result of alterations in mRNA stability (Arkins et al., 1995a,b,c). Activation of the human macrophage-like cell line U937 with either the Ca^{2+} ionophore calcimycin (A23187) or phorbol acetate, results in the up-regulation of IGF-I gene transcription, as assessed by nuclear run-

off assays (Nagaoka et al., 1990). These actions require ongoing intermediate protein synthesis. On the other hand, steady-state levels of cytoplasmic IGF-I mRNA declined with macrophage activation, and this effect could be blocked with an inhibitor of protein kinase C. The release of IGF-I protein from activated cells is rapid and resistant to protein synthesis inhibition, suggesting that the released protein comes from a substantial preformed pool of molecules in macrophages (Nagaoka et al., 1990). IL-4 and IL-13 induce IGF-I production in mouse macrophages through the enhancement of gene transcription (Wynes and Riches, 2003). Moreover, interferon γ could block the actions of these Th2 cytokines. STAT6 mediated the actions of IL-4 and IL-13, whereas STAT1 was necessary for the attenuating effects exerted by interferon γ (Wynes and Riches, 2003). In a growth factor withdrawal model of lung fibrosis, IL-4-induced macrophage-derived IGF-I protects CCL39 myofibroblasts from apoptosis, an action attenuated with IGF-I-specific neutralizing antibodies (Wynes et al., 2004). Macrophages and monocytes express receptors for advanced glycosylation end-products. When these receptors become activated, IGF-I mRNA is up-regulated in monocytes and IGF-I protein is released (Kirstein et al., 1992). Levels of IGF-I mRNA and IGFBP-4 increased in a time-dependent manner in cultured bone marrow-derived murine macrophages subjected to macrophage colony stimulating factor-1. These experimental conditions promote cell proliferation and differentiation (Long et al., 1998). Those studies suggested that IGFBP-4 might exert a modulating effect on IGF-I-dependent macrophage differentiation and proliferation.

D. Neutrophils and Other Granulocytes

Other members of the immune system also seem to share important relationships with IGF-I and its signaling pathway. Notable among these are neutrophils, which serve as important effector cells in innate immunity. They function as an early defense against microorganisms. Neutrophils undergo spontaneous apoptosis, a process that seems to be slowed by IGF-I (Kooijman et al., 2002). In a later study, these same investigators demonstrated that IGF-I can block Fas-mediated apoptosis (Himpe et al., 2008). The pro-survival effects of the growth factor are mediated through the phosphatidylinositol-3 kinase pathway. Moreover, the presence of cytokines failed to alter the antiapoptotic actions of IGF-I, suggesting that they may play a dominant role, even within the context of active inflammation. In contrast, IGF-I seems to attenuate the impact of stress on mouse gastric mucosal injury by inhibiting neutrophil activation (Zhao et al., 2009). IGF-I mRNA is developmentally regulated in mononuclear phagocytic cells (Arkins et al., 1993, 1995a,b,c).

V. Implications of Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-I Receptor in Immune Modulation

What does the realization that a growth factor pathway can influence immune function teach us about integrative human biology? Why should molecules playing dominant roles in the regulation of metabolism, growth, and development also influence the function of the immune system? What advantage to the organism does such integration provide with respect to the overall energetic economy, survival, and host defense? Is nature "double-dipping" and using a well traveled set of signaling pathways for multiple unrelated purposes? Or does the logic emerging from a better understanding of evolution help us to identify the pressures that are driving such an overlap?

Great wisdom may be found in this sharing of common pathways. First, growth factors have now been implicated in normal and pathological wound healing and in tissue remodeling under a variety of clinical circumstances. The IR and its downstream signaling targets, including Akt, are now thought to regulate the metabolic activity of T cells (Frauwirth and Thompson, 2004). Insulin itself has been shown to exhibit a set of chemoattractant properties (Berman and Center, 1987). Phytohemagglutinin-activated human T cells are 100-fold more responsive to porcine insulin than are resting lymphocytes. Moreover, both $CD4^+$ and $CD8^+$ cells behave identically when treated with insulin (Berman and Center, 1987). PI3 kinase mediates many of the actions of T-cell costimulatory molecules including CD28. Activation of CD28, with TCR, can coordinately up-regulate glucose availability and metabolism and can therefore accommodate the increased energetic needs associated with cell activation. A specific role for the IGF-I pathway in influencing metabolic activity in lymphocytes has yet to be explored, but its similarities to that of insulin suggest that both may modulate energy turnover in immune responses.

Taken in aggregate, these studies tell us that substantial cross-talk occurs between a complex array of pathways that have proven integral to energy turnover, growth, and development. These pathways, however, seem to determine, at least in part, the amplitude and quality of immune responses. A thread common to these biological functions relates to the high energetic costs of each. It seems logical and even likely, therefore, that a selection advantage underlies the coordination between these aspects of survival. However, because these components are overlapping, interrupting any one of them might have untoward consequences and thus might limit therapeutic strategies targeting these pathways. Therein lies the particular attraction associated with the therapeutic targeting of receptors such as IGF-IR. These lie upstream from the intermediate signaling kinases that participate in multiple signaling pathways.

VI. Insulin-Like Growth Factor-I, Insulin-Like Growth Factor-I Receptor, and Autoimmunity

The constellation of autoimmune diseases continues to represent a particularly vexing group of maladies that are tied together by common etiologic features with uncertain identities. They are thought to emerge from both genetic and environmental factors. Genetic predisposition to autoimmunity was demonstrated nearly 40 years ago by Vladutiu and Rose (1971). There is reason to suspect that shared susceptibility genes participate in many, because they cluster in families. Moreover, patients often manifest multiple diseases. Most but not all exhibit strong female gender predilection (Fairweather et al., 2008). Moreover, the general features of autoimmunity occurring in men and women differ. At the center of these diseases are many of the same pathological features associated with other chronic processes in which inflammation gives way to tissue remodeling. The findings to date concerning the putative role of IGF-I/IGF-IR in regulating immune function have begun to suggest its potential involvement in autoimmunity. Specifically, IGF-I influences the physiological behavior of lymphocytes and other professional immune cells through its activation of IGF-IR. Responses to growth factors and the display of their receptors at relatively high levels could underlie the participation of these cells in chronic inflammatory disease. T cell activation seems to be coupled to increased IGF-I responses (Berman and Center, 1987). Thus, a potential involvement of the IGF-I pathway might help explain several aspects of human autoimmunity. These lines of evidence will be reviewed.

A. Systemic Inflammation

Several potential connections have been made recently between the loss of tolerance to self-antigens and the actions of IGF-I. Of particular interest is the potential for IGF-I to influence the functions and regulation of inflammatory effector cells, particularly professional phagocytes. For instance, cathelicidin hCAP-18/LL37, an antimicrobial peptide involved in innate immunity, can be detected in alveolar macrophages, neutrophils, and macrophages after infection with *Mycobacterium tuberculosis* (Drop et al., 1992). It is constitutively expressed or can be induced in these cells through toll-like receptors 9, 4, and 2 (Rivas-Santiago et al., 2008). LL-37 complexes with DNA and RNA and, in doing so, elicits immune responses to microbial nucleic acids. Cathelicidin hCAP-18/LL37 is also expressed by A549 epithelial cells and, in this context, has been implicated in the pathogenesis of psoriasis (Lande et al., 2007) through a mechanism involving plasmacytoid dendritic cells becoming activated to host DNA. Human keratinocytes express LL-37 as well as human β-defensin 3, neutrophil gelatinase-associated lipocalin, and secretory leukocyte protease inhibitor in response to IGF-I and TGF-α (Sørensen et al., 2003). Is it thus possible that the upregulation of LL-37 by IGF-I and the pro-inflammatory cytokine, TGF2α, elicits autoimmune responses through inappropriate reactivity to host DNA or RNA? Could these insights provide important clues regarding IGF-I activation of innate immunity during tissue injury? LL-37 might also possess inhibitory activity against proteases, potentially resulting in a breakdown of immune tolerance.

B. Graves' Disease

Graves' disease has recently been examined as potentially involving the IGF-I/IGF-IR pathway. It represents an autoimmune syndrome with multiple components, the most frequent of which involves thyroid gland overactivity and enlargement (Davies, 1996). In addition, the tissues surrounding the eye, called the orbit, become activated in a substantial fraction of patients with Graves' disease in a process known as thyroid-associated ophthalmopathy (TAO). The activation of orbital fibroblasts seems to be at the center of TAO pathogenesis. These cells have a phenotype distinct from fibroblasts inhabiting other connective tissues (Smith et al., 1995, 2002).

Graves' disease is a prototypic example of antibody-driven autoimmunity. Central to promoting the hyperthyroid state and thyroid enlargement in this disease are activating antibodies directed against the thyroid-stimulating hormone receptor (thyrotropin receptor or TSHR) (Zakarija et al., 1988). These antibodies, termed thyroid-stimulating immunoglobulins, override the trophic control of thyroid function normally imposed by the hypothalamic/anterior pituitary axis through its elaboration by thyrotrophs of TSH. TSH ordinarily acts as the dominant regulator of the biosynthetic activities of thyroid epithelial cells. This axis is regulated by a sensitive feedback loop that responds to the circulating levels of the thyroid hormones thyroxine and triiodothyronine. Adequate levels of these hormones turn off the production and release of TSH in states of thyroid hormone sufficiency. TSHR represents a seven membrane-spanning G protein-coupled receptor (Parmentier et al., 1989). Its expression was originally thought to be limited to the thyroid epithelium. More recently, it has been found to be displayed on fat cells in several connective tissue depots, and unexpectedly by $CD11b^-$ bone marrow cells in mice (Bell et al., 2000; Wang and Klein, 2001; Klein, 2003). Of potential importance to the pathogenesis of TAO is the demonstration of TSHR mRNA in orbital tissues (Feliciello et al., 1993) and by orbital fibroblasts (Heufelder et al., 1993).

An intriguing relationship between thyroid function and IGF-I was first recognized more than 20 years ago, when the rat clonal thyroid epithelial cell line FRTL-5 was found to exhibit far greater responses to TSH in the presence of either insulin or IGF-I in the culture medium (Tramontano et al., 1986, 1987, 1988a,b). Ingbar et

al. found that both IGF-I and TSH could enhance FRTL-5 cell proliferation and DNA synthesis in a concentration-dependent manner and that the actions of these agents were synergistic (Tramontano et al., 1986). In contrast, IGF-I does not influence TSH-dependent cAMP generation in these FRTL-5 cells (Tramontano et al., 1986, 1987, 1988a,b). When IGF-I is replaced by insulin, equivalent effects are seen. Both insulin and IGF-I down-regulate major histocompatibility complex class I gene expression (Giuliani et al., 2006). These growth factors depress the activity of the intact PD1 gene promoter but up-regulate those of mutant promoter constructs lacking the tissue-specific region but retaining a gene enhancer sequence. Moreover, IGF-I and insulin facilitate the interactions between promoter regions with transcription factors, including activator protein-1 and nuclear factor-κB (Giuliani et al., 2006). Clément et al. (2001) demonstrated that the conditional overexpression of both IGF-I and IGF-IR in the thyroids of double-transgenic mice increases thyroid gland weight and follicular lumen volumes in vivo. A number of reports have suggested that substantial overlap exists between the signaling elicited by TSH and IGF-I in thyroid epithelium. Specifically, several signaling events downstream from TSHR are mediated through the PI3 kinase pathway and involve activation of the Akt/FRAP/mTOR/p70^{s6k} pathway (Cass and Meinkoth, 1998; Park et al., 2000a,b, 2005). This pathway mediates a number of the growth-promoting actions of IGF-I in many responsive cell types. In addition, it regulates the translation of proteins encoded for by preformed mRNAs containing an oligopyrimidine tract at the transcriptional start site (Jefferies et al., 1997).

It has been proposed that IGF-IR and TSHR might participate together in the pathogenesis of Graves' disease through their physical and functional interactions. Activation of each receptor results in the utilization of common downstream signaling pathways. Specifically, TSHR and IGF-IR seem to act in concert to regulate discrete metabolic activities within the thyroid, including cellular proliferation and apoptosis. Recently, a report suggested that TSHR and IGF-IR colocalize in orbital fibroblasts and in human thyroid epithelial cells in culture (Tsui et al., 2008). Moreover, these investigators found that both IGF-IRβ and TSHR could be pulled down by antibodies specific for either protein. It is noteworthy that that report also suggested that interrupting IGF-IR signaling by incubating cells with a blocking monoclonal antibody directed against IGF-IRα (1H7) could attenuate the activation of Erk provoked by TSH (Tsui et al., 2008). Those findings imply a functional complex comprising TSHR and IGF-IR and that IGF-IR transactivation could mediate at least some aspects of TSHR signaling. Whether this relationship between the two receptors in any way relates to the pathogenesis of Graves' disease remains uncertain.

Activation of the orbital connective tissue in TAO is characterized by often intense inflammatory responses involving T-, B-, and mast-cell infiltration of orbital tissues and their dramatic remodeling (Prabhakar et al., 2003). Although the antibodies generated against the TSHR help explain the aberrant growth of thyroid tissue and its overproduction of thyroid hormone, any role for that receptor and the activating antibodies generated against it in TAO remains incompletely understood. Reconciling the tissue reactivity in the orbit with the thyroid pathology on the basis of a one-antigen/one antibody paradigm has been difficult to accomplish. Other antigens besides TSHR have been examined for their potential involvement in TAO. Among them, the IGF-I pathway was first implicated in studies examining whether IgG from patients with Graves' disease (GD-IgG) could displace ^{125}I-IGF-I from the surface of human fibroblasts (Weightman et al., 1993). Those studies demonstrated a dose-dependent competition with radiolabeled IGF-I from fibroblast surfaces. Although this study was incomplete in that it neglected to explore the identity of the binding site(s), it broke new ground in implicating the IGF-I pathway in Graves' disease. The study also fell short of characterizing whether GD-IgG could either block occupancy or activate the receptor. The basis for loss of peripheral immune tolerance to IGF-IR underlying the generation of the IGF-IR-activating antibodies in Graves' disease remains unknown.

More recently, a study has demonstrated increased density of IGF-IR on the surface of orbital fibroblasts from patients with Graves' disease (Pritchard et al., 2003). In addition, the frequency of fibroblasts expressing the receptor protein is also elevated above that found in control cultures derived from healthy tissue. The levels of IGF-IR are approximately 3- to 4-fold higher than those found in control fibroblasts. Moreover, GD-IgGs could activate fibroblasts from patients with Graves' disease from several connective tissue depots (Pritchard et al., 2003). These include orbital fat, neck skin, abdominal skin, and pretibium. When treated with GD-IgG or with IGF-I, these fibroblasts expressed two powerful T-cell chemoattractants, namely IL-16 and RANTES (Pritchard et al., 2002). IL-16 specifically targets CD4$^+$-bearing T cells and therefore serves as a chemoattractant for only one subset of lymphocytes (Klimiuk et al., 1999). It is expressed by both CD4$^+$ and CD8$^+$ lymphocytes, epithelial cells, fibroblasts, and mast cells (Center et al., 1997, Sciaky et al., 2000). The mechanisms involved in the synthesis of IL-16 and regulation of its expression vary among these cell types. In fibroblasts, relatively high levels of untranslated IL-16 mRNA can be detected under basal culture conditions. Cell activation with IGF-I leads to the caspase-3-dependent production of IL-16 protein (Zhang et al., 1998). RANTES, a C-C chemokine, is also expressed by several cell types and exerts its biological actions through multiple G protein-coupled receptors, including CCR4 and CCR5

(Schall et al., 1990). RANTES targets several subpopulations of lymphocytes. Both IL-16 and RANTES have been implicated previously in other autoimmune diseases (Klimiuk et al., 1999; Simchen et al., 2000) and elevated levels of both have been detected in the general circulation of patients with chronic inflammatory disease (Blaschke et al., 2001; Christodoulakos et al., 2007).

The induction of IL-16 by IGF-I in fibroblasts from patients with Graves' disease involves the activation of the Akt/mTOR/FRAP/p70^{s6k} pathway and can be attenuated with rapamycin, a macrolide exhibiting both antifungal and immunosuppressive activities (Pritchard et al., 2002). Rapamycin specifically targets mTOR, one of the terminal kinases coupling mitogenic stimulation to the serine/threonine phosphorylation of the eukaryotic initiation factor (eIF)-4E-binding protein, PHAS-I (Pritchard et al., 2002). Physiological concentrations of glucocorticoids also block the induction by IGF-I of IL-16 and RANTES (Pritchard et al., 2002). In contrast, the induction of RANTES seems unaffected by rapamycin and, unlike that of IL-16, involves IGF-I actions mediated at the pretranslational level (Pritchard et al., 2002). The mechanism for IGF-I and GD-IgG induction of these chemoattractants remains incompletely described. The antibody(s) was found to bind to IGF-IR (Pritchard et al., 2003). An IGF-IR blocking monoclonal antibody, 1H7, completely abrogates the signaling and induction of IL-16 and RANTES-dependent T-cell migration activity generated by fibroblasts treated with either IGF-I or GD-IgG (Fig. 4) (Pritchard et al., 2003). Moreover, transfection of TAO orbital fibroblasts with a mutant dominant-negative IGF-IR, designated 486 STOP (Reiss et al., 2001), could also block IL-16 and RANTES synthesis resulting from the actions of IGF-I and GD-IgG (Fig. 5). Subsequently, GD-IgG and IGF-I were found to also induce the synthesis of hyaluronan in orbital fibroblasts from donors with TAO (Smith and Hoa, 2004). It is noteworthy that control orbital fibroblasts from persons without autoimmune diseases failed to respond to either IGF-I or GD-IgG (Pritchard et al., 2002, 2003; Smith and Hoa, 2004). This same pattern of IL-16 and RANTES induction by IGF-I was subsequently described in cultured primary human thyroid epithelial cells (Gianoukakis et al., 2006). Unlike control fibroblasts, thyrocytes from donors without Graves' disease or other autoimmune thyroid processes also responded to IGF-I and GD-IgG. What has emerged from these studies conducted in cultured fibroblasts and thyrocytes is the concept that IGF-IR might play a critical role in activating T-cell trafficking signals in infiltrated tissues.

Finding more numerous IGF-IR$^+$ fibroblasts with increased receptor density raised questions concerning whether other cell types might also overexpress the protein. Moreover, this abnormal pattern of expression could help explain loss of peripheral immune tolerance to IGF-IR. Examination of circulating T cells from patients with Graves' disease has disclosed an over-abundance of IGF-IR$^+$ lymphocytes (Fig. 6) (Douglas et al., 2007). The frequency of receptor-harboring T cells was found to be increased from controls, in which 15 ± 3% of CD3$^+$ T cells expressed the receptor compared with 48 ± 5% (n = 33, $p \leq 10^{-8}$ versus control) among those from patients. This divergence does not vary with the stage of the disease, treatment, or duration of illness. The phenotypic skew toward IGF-IR$^+$ T cells segregates unequally among several lymphocyte subsets. It is noteworthy that CD4$^+$CD45RO$^+$IGF-IR$^+$ and CD8$^+$CD45RO$^+$IGF-IR$^+$ memory T cell populations are extraordinarily rare in healthy persons without the disease (<5%) (Fig. 7). However, they become predominant among memory T cells in donors with Graves' disease, whether or not the patient manifests clinically important TAO (Douglas et

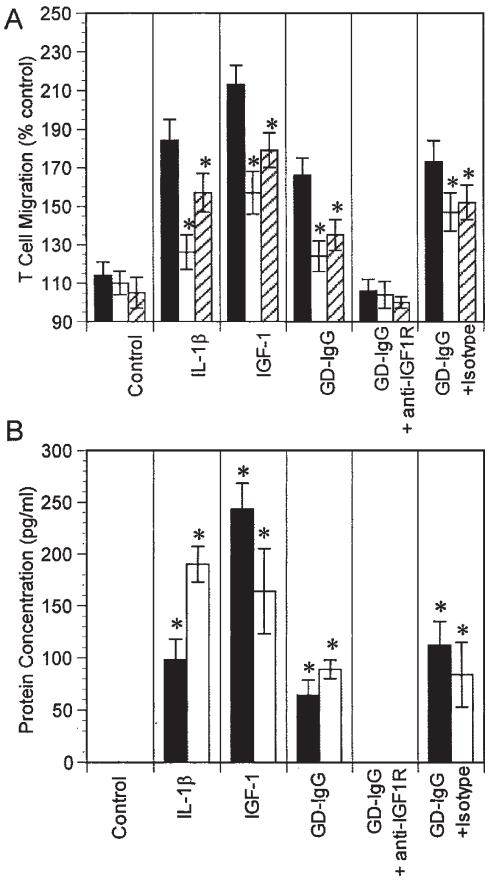

FIG. 4. The effects of IL-1β, IGF-I, and GD-IgG, without or with anti-IGF-IR antibody 1H7, on T-cell chemotactic activity (A) and IL-16 (■) and the RANTES (□) protein expression (B) in fibroblasts from donors with GD. Cultures were treated with IL-1β (10 ng/ml), IGF-I (10 nM), and GD IgG (100 ng/ml), without or with antibody 1H7 (5 μg/ml) for 24 h, then the media were subjected to T-cell migration assays or specific enzyme-linked immunosorbent assays. Samples used for chemotaxis analysis were then treated with no Ab (■) or anti-IL-16 (clone 14.1, 5 μg/ml; □) or anti-RANTES (5 μg/ml; ▨) neutralizing antibodies, as indicated. Migratory data are expressed as a percentage compared with unstimulated (random) migration, which is designated 100%. *, statistically different migration in the presence of neutralizing antibodies (A) or protein production (B) at the 5% confidence level. [Reprinted from Pritchard J, Han R, Horst N, Cruikshank WW, and Smith TJ (2003) Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway. *J Immunol* **170:**6348–6354. Copyright © 2003 The American Association of Immunologists, Inc. Used with permission.]

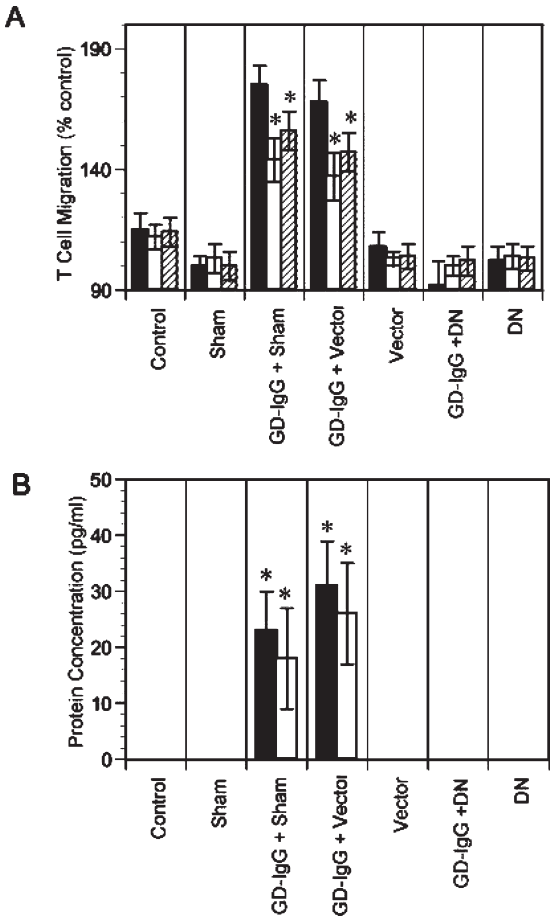

FIG. 5. Expression of a DN mutant IGF-IR in GD fibroblasts can block the effects of GD-IgG on T-cell chemoattractant activity (A) and IL-16 (■) and RANTES (□) protein expression (B). Confluent cultures of fibroblasts from a patient with GD were transiently transfected with a plasmid containing the dominant-negative mutant IGF-IR designated 486/STOP or with empty vector (as control). Cultures were then treated with GD-IgG (100 ng/ml) or nothing (control) for 24 h. Media were collected and analyzed for T-cell migratory activity without (■) or with either anti-IL-16 (□) or anti-RANTES (▨) neutralizing antibodies (5 μg/ml) or for IL-16 and RANTES protein expression. The migratory data are expressed as a percentage compared with unstimulated (random) migration, which is designated 100%. *, statistically different migration in the presence of neutralizing antibodies (A) or protein production (B) at the 5% confidence level. [Reprinted from Pritchard J, Han R, Horst N, Cruikshank WW, and Smith TJ (2003) Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway. *J Immunol* **170:**6348–6354. Copyright © 2003 The American Association of Immunologists, Inc. Used with permission.]

al., 2007). In some patients, more than 95% of $CD8^+CD45RO^+$ T cells display IGF-IR. Unlike TAO-derived orbital fibroblasts, in which cell-surface IGF-IR densities are 3- to 4-fold higher than those on control cells, receptor levels on T cells from patients with Graves' disease are similar to those found on control lymphocytes (Douglas et al., 2007). As with fibroblasts, T cells bearing the $IGF\text{-}IR^+$ phenotype exhibit preferential resistance to apoptosis and a growth advantage in vitro. Orbital T cells from those patients manifesting severe TAO also exhibit the skewed phenotype. Studies examining the phenotype of peripheral and orbital B cells from these patients suggest that they too are skewed toward the $IGF\text{-}IR^+$ phenotype. Of the B cells from donors with Graves' disease ($n = 30$), 34 ± 4% (mean ± S.E.) display IGF-IR, whereas the receptor was detected on 9 ± 3% of B cells from 24 control donors (Fig. 8) (Douglas et al., 2008). Analogous to T cells, the phenotype of B cells with the $IGF\text{-}IR^+$ skew also seems durable in patients observed over many months. Moreover, it seems to remain, even in those whose diagnosis was made years before their participation in the study. IGF-I treatment promotes B cell survival in vitro and seems to act synergistically with CpG to enhance total IgG production (Fig. 9). EBV-transformed $IGF\text{-}IR^+$ B cells consistently produced anti-TSHR antibodies, suggesting that the skew toward receptor expression might underlie autoantibody production. Future examination of other phenotypic attributes displayed by $IGF\text{-}IR^+$ B cell subsets might prove enlightening. These include determining the impact of IGF-IR display and activation on antigen presentation, cytokine production, and T-cell-dependent B-cell activation.

Both genetic and acquired factors are known to contribute to the pathogenesis of Graves' disease (Brix et al., 1998). Much investigation has focused on the identification of a gene locus or multiple loci that could help explain susceptibility to the disease. A number of candidates have been found (Ban and Tomer, 2005). Nevertheless, no single gene seems likely to explain the pattern of inheritance of Graves' disease in large cohorts of patients and their families. Indeed, substantial evidence supports the importance of acquired factors in disease development layered on to as yet unidentified genetic determinants (Prummel et al., 2004). These conclusions rest on data from population-based twin studies, especially those using the Danish Twin cohort. Examination of monozygotic twins discordant for Graves' disease suggests that 70% of the cause of the disorder comes from environmental aspects of disease acquisition (Brix et al., 1998). Douglas et al. (2009) found that the majority of clinically healthy, unaffected monozygotic twins in discordant pairs failed to exhibit an $IGF\text{-}IR^+$ skewed population of circulating T and B cells compared with their twin with Graves' disease. The study replicated observations made earlier by the same authors in a separate North American cohort of patients with sporadic Graves' disease (Douglas et al., 2007, 2008). It is noteworthy that this recent study demonstrates that the more frequent display of IGF-IR on T and B cells, associated with Graves' disease, seems to derive from acquired rather than genetic factors. Thus, for the first time in the context of Graves' disease, a discrete cellular attribute might be ascribed to acquired factors. Whether the skew toward $IGF\text{-}IR^+$ phenotype in B and T cells might be shared with other chronic autoimmune diseases or is specific to Graves' disease remains to be determined.

These findings identify IGF-IR as a potentially attractive target for interrupting early disease-related processes. That strategy would have the goal of preventing

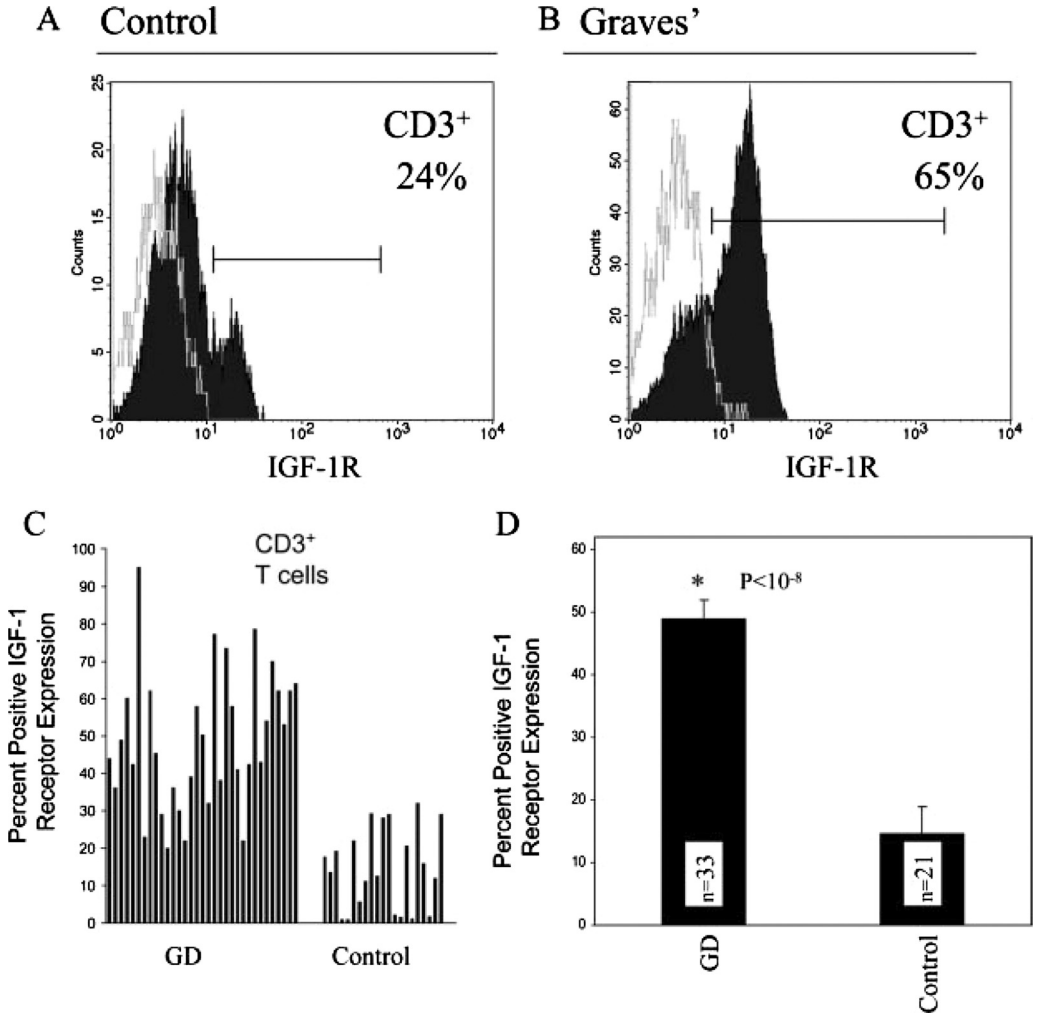

FIG. 6. Increased fraction of peripheral blood T cells from patients with GD display IGF-IR compared with those from control donors. Peripheral blood mononuclear cells were stained with anti-CD3 and IGF-IR antibodies and subjected to multiparameter flow cytometry. A and B, the open histograms represent staining with isotype control antibodies. Data are derived from single, representative samples from each source. C, fraction of IGF-IR$^+$ CD3$^+$ T cells from individual patients with GD and control donors. D, analysis of IGF-IR display in T cells from the aggregate of multiple patients with GD and control donors; 48 ± 4% GD T cells (n = 33) display IGF-IR compared with 15 ± 3% control T cells (n = 21; $p < 10^{-8}$). Data are expressed as mean ± S.E. [Reprinted from Douglas RS, Gianoukakis AG, Kamat S, and Smith TJ (2007) Aberrant expression of the IGF-1 receptor by T cells from patients with Graves' disease may carry functional consequences for disease pathogenesis. *J Immunol* **178:**3281–3287. Copyright © 2007 The American Association of Immunologists, Inc. Used with permission.]

local trafficking of lymphocyte subsets responsible for driving tissue reactivity and remodeling. Such an approach could be based on the use of antibodies that block receptor binding or small molecules that interfere specifically with IGF-IR phosphorylation or its interaction with relevant docking proteins. Whatever treatment approach proves most rewarding, IGF-IR has emerged as a potentially important therapeutic target for attenuating connective tissue activation in Graves' disease. This is especially true of those disease manifestations that remain ineffectively treated, such as TAO. Early experiences with the anti-IGF-IR blocking antibodies currently being examined for treating various forms of cancer suggest that many of these might prove well tolerated. The absence of complete and robust preclinical models of Graves' disease (Baker et al., 2005) in which to test the efficacy of this approach imposes a considerable obstacle to efficiently identifying new therapies.

C. Other Autoimmune Diseases

A number of autoimmune diseases have been superficially examined for their potential association with abnormalities in the IGF-I/IGF-IR pathway. A substantial link between this pathway and the pathogenesis of these diseases has yet to be established. However, a limited body of information thus far generated suggests that IGF-I, its receptors, and its binding proteins might ultimately prove relevant to the mechanistic underpinnings of several allied diseases. The insights recently generated concerning the involvement of IGF-IR in Graves' disease would seem to make similar efforts in these other autoimmune processes potentially rewarding.

1. Diabetes Mellitus. Like the other common human autoimmune diseases, type 1 DM represents a convergence of multiple genetic and environmental factors (Eisenbarth et al., 1994). Its pathogenesis is linked to

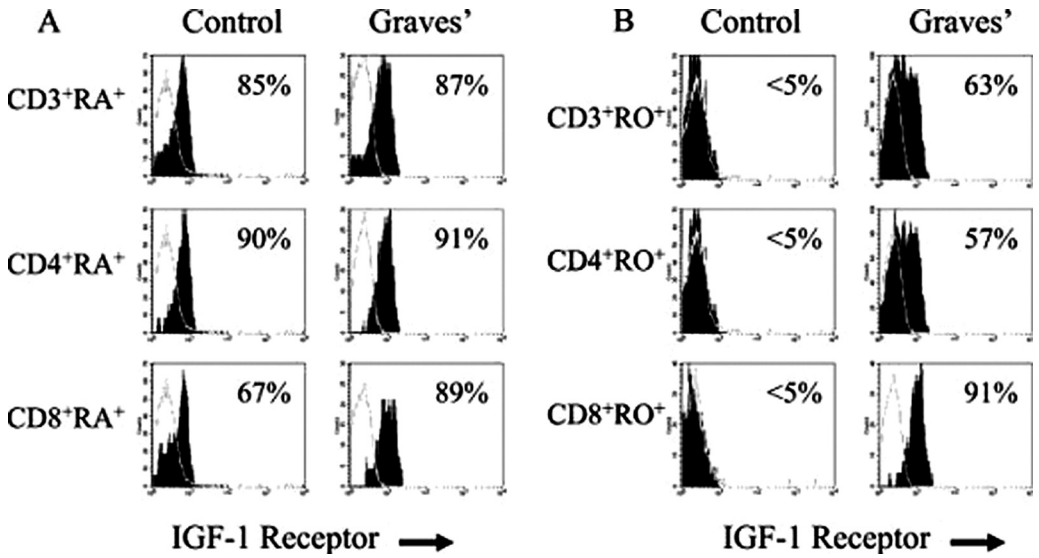

FIG. 7. Disproportionate IGF-IR$^+$CD45RO$^+$ memory T cells from patients with GD. The fraction of CD3$^+$, CD4$^+$, and CD8$^+$ T lymphocytes expressing IGF-IR was determined using multiparameter flow cytometry by gating on populations of CD3$^+$, CD4$^+$, or CD8$^+$, CD45RA$^+$, or CD45RO$^+$ T cells and is represented as a histogram (filled) compared with isotype controls (open). A, naive CD45RA$^+$ lymphocytes from a patient with GD and a control donor demonstrate a similar, frequent display of IGF-IR. B, the fraction of memory CD45RO$^+$ lymphocytes expressing IGF-IR is dramatically greater in lymphocytes from a patient with GD compared with control. GD CD8$^+$CD45RO$^+$ T lymphocytes uniformly express IGF-IR, compared with infrequent control CD8$^+$CD45RO$^+$ cells. T-cell expression of IGF-IR was representative of our aggregate observations. [Reprinted from Douglas RS, Gianoukakis AG, Kamat S, and Smith TJ (2007) Aberrant expression of the IGF-1 receptor by T cells from patients with Graves' disease may carry functional consequences for disease pathogenesis. *J Immunol* **178:**3281–3287. Copyright © 2007 The American Association of Immunologists, Inc. Used with permission.]

innate immunity and inflammatory dysfunction of β islet cells of the pancreas (Eizirik et al., 2009). At least 10 genetic loci have been associated with the disease, including cytotoxic T lymphocyte-associated protein 4, human leukocyte antigen class II genes, IL-2 receptor α, protein tyrosine phosphatase nonreceptor 22, and interferon-induced helicase C domain 1. Although most of these have been extensively examined, additional candidates continue to be identified (Todd et al., 2007). A number of polymorphisms of the *IGF-I* gene have been identified, and the implications of these genetic variations to the development of diabetes are currently being explored (Vella et al., 2008).

A number of aspects of both normal and pathological endocrine pancreatic function have been examined with

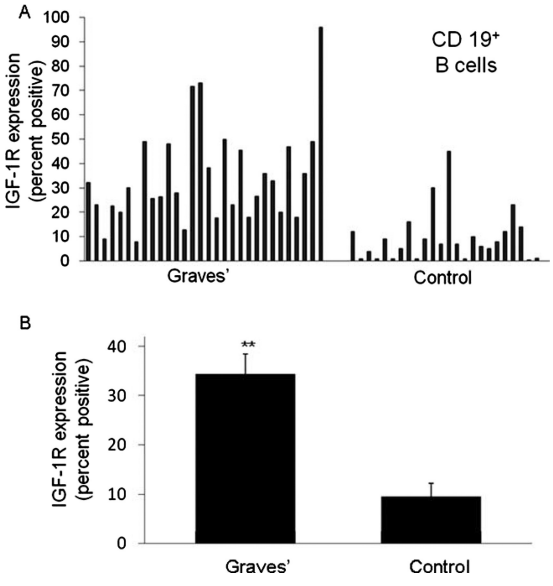

FIG. 8. A disproportionate fraction of peripheral blood B cells from 30 patients with GD express IGF-IR compared with that found in 24 control donors. A, individual data sets demonstrating fractional IGF-IR$^+$ B cells. B, analysis of IGF-IR display in B cells as an aggregate of multiple patients with GD versus control donors [34 ± 4% IGF-IR$^+$ B cells (mean ± S.E., $n = 30$) versus 9 ± 3% IGF-IR$^+$ control B cells ($n = 24$)]. Data are expressed as means ± S.E. (**, $p < 10^{-6}$). [Reprinted from Douglas RS, Naik V, Hwang CJ, Afifiyan NF, Gianoukakis AG, Sand D, Kamat S, and Smith TJ (2008) B cells from patients with Graves' disease aberrantly express the IGF-1 receptor: implications for disease pathogenesis. *J Immunol* **181:**5768–5774. Copyright © 2008 The American Association of Immunologists, Inc. Used with permission.]

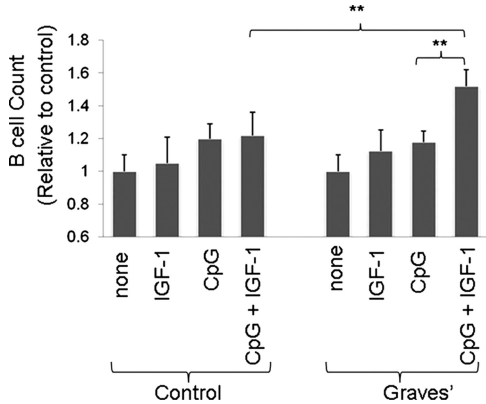

FIG. 9. IGF-I potentiates B-cell expansion when added together with a concentration of CpG, yielding a submaximal response. Number of B cells was assessed after 5 days in culture with CpG (2 μg/ml) and IGF-I (10 nM) as single agents or in combination. Data were derived from five independent experiments (mean ± S.E.; **, $p < 0.02$). [Reprinted from Douglas RS, Naik V, Hwang CJ, Afifiyan NF, Gianoukakis AG, Sand D, Kamat S, and Smith TJ (2008) B cells from patients with Graves' disease aberrantly express the IGF-1 receptor: implications for disease pathogenesis. *J Immunol* **181:**5768–5774. Copyright © 2008 The American Association of Immunologists, Inc. Used with permission.]

regard to the insulin and IGF-I signaling pathways as regulators of immune function and dysfunction. Xuan et al. (2002) reported that tissue-specific, conditional mutagenesis of IGF-IR, resulting in its silence, failed to alter β cell mass. On the other hand, ablation of IGF-IR signaling resulted in an age-dependent decline in glucose and arginine-dependent insulin release, underscoring the importance of this receptor in normal pancreatic function. Therefore, carbohydrate intolerance might result from altered function of the IGF-I pathway. In the Goto-Kakizaki rat model of type II DM, defective IGF-II and IGF-IR expression in the embryonic pancreas results in diminished β-cell mass and precedes hyperglycemia (Calderari et al., 2007). Insulin itself exhibits substantial chemotactic activity to T cells (Berman and Center, 1987). In phytohemagglutinin-activated T cells, the chemotactic response to insulin was enhanced compared with that exhibited by resting lymphocytes. Moreover, $CD4^+$ and $CD8^+$ cells responded identically. The kinetics suggested that high-affinity IR was mediating this response (Berman and Center, 1987). T cells express and display IR after activation and treatment with insulin enhances intermediary metabolism (Krug et al., 1972; Helderman and Strom, 1977), progression through the cell cycle (Snow et al., 1980; Helderman, 1981; Kumagai et al., 1981), and an up-regulated effector function (Brown et al., 1983). Thus, it is entirely possible that elevated serum insulin levels, resulting from states of insulin resistance such as those found in type II DM, might result in a substantial and direct impact on T cells. This is true of lymphocytes in the circulation as well as those infiltrating the pancreas.

IGF-I and IGF-II play integral roles in the development and function of β-islet cells, which produce both proteins and respond to them through IGF-IR (Van Schravendijk et al., 1987; Zhang et al., 1997). IGF-I promotes islet growth, an action mediated by IRS-1 (Bonner-Weir and Smith, 1994). Conditional knockout of IGF-IR in β cells results in elevated blood insulin levels and glucose intolerance but fails to influence β-cell development or mass (Kulkarni et al., 2002). In contrast, global interruption of IGF-IR, such as that found in *Igf1r*(−/−) mice, results in reduced β-cell mass (Withers et al., 1999). IGF-I can protect against diabetes in a NOD mouse model, where autoreactive T cells were adoptively transferred and donors treated with either saline or the growth factor (Bergerot et al., 1995). IGF-I reduced the incidence of diabetes, abrogated insulitis, and increased the abundance of intact islets. Transgenic mice, the β-islet cells of which overexpress IGF-I, exhibit enhanced recovery from cytotoxicity after treatments with streptozotocin (Fig. 10) (George et al., 2002). The Fas and β2-microglobulin hyperexpression and lymphocytic infiltration observed in interferon β single trans-

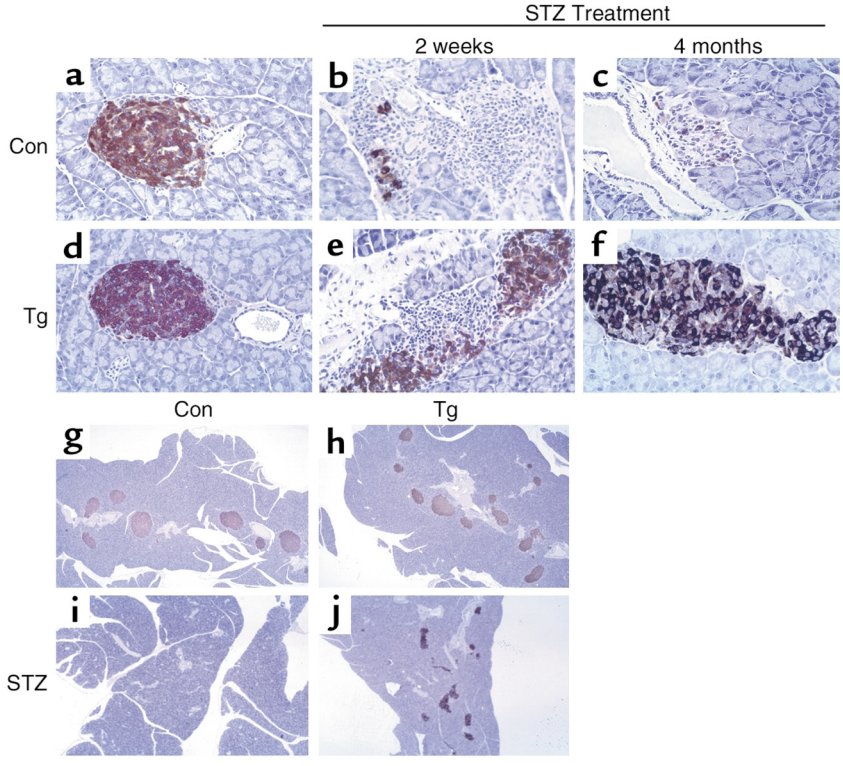

FIG. 10. Analysis using immunohistochemistry of insulin expression by islets from N4 CD-1 mice. a–f, pancreata from 2-month-old, untreated nontransgenic (Con) (a) and transgenic (Tg) (d) mice, nontranstgenic (b) and transgenic (e) mice treated with streptozotocin (STZ) for 2 weeks, whereas other nontransgenic (c) and transgenic (f) mice were treated for 4 months. Magnification, 400×. g–j, analysis of 6-month-old, untreated nontransgenic (g) and transgenic (h) mice and nontransgenic (i) and transgenic (j) mice treated for 4 months with STZ. Magnification, 40×. [Reproduced from George M, Ayuso E, Casellas A, Costa C, Devedjian JC, and Bosch F (2002) β Cell expression of IGF-I leads to recovery from type 1 diabetes. *J Clin Invest* **109:**1153–1163. Copyright © 2002 American Society for Clinical Investigation. Used with permission.]

genic animals was abrogated in double-transgenic animals harboring β cells overexpressing both IGF-I and interferon β (Casellas et al., 2006). Moreover, the susceptibility of the single transgenic animals to streptozotocin-induced diabetes was markedly reduced. Administration of IGF-I, either as a free molecule or complexed with IGFBP3, reduced the severity and delayed the onset of diabetes in NOD mice (Chen et al., 2004). This protective effect is mediated by increased CCL4 production and dampened CCL3 expression. Within the β cell, IGF-I activates Akt signaling, resulting in enhanced proliferation and resistance to apoptosis. Human β cells transfected with the IGF-I gene also exhibit resistance to apoptosis induced by IL-1β (Giannoukakis et al., 2000).

Because of its antiapoptotic effects on β islet cells, and its promotion of insulin secretion (Kido et al., 2002; Xuan et al., 2002), IGF-I has been proposed as a potential therapy for DM, especially when associated with insulin resistance (Murphy, 2006). Its administration has been shown to result in enhanced insulin sensitivity and improved glycemic control in both type I and II DM (Clemmons et al., 2000, 2005). Pennisi et al. (2006), using MKR mice in which defective IGF-IR dimerizes with endogenous IGF-IR and IR (Le Roith et al., 2002), have reported that administration of IGF-I results in reduced glucose levels. This effect can be attributed to reduced endogenous glucose production rather than enhanced whole-body insulin sensitivity. The findings of this and similar studies have reinvigorated consideration of IGF-I and its analogs in the therapy of DM, a strategy largely abandoned because of the potential side effects associated with its administration.

2. Crohn's Disease. Inflammatory diseases affecting the gut continue to plague society. The etiologies of the two principal forms, ulcerative colitis and Crohn's disease, remain enigmatic, as does the pathogenic relationship between the two. Both are believed to represent an overdetermined immune reactivity to commensal bacteria in persons with a susceptibility to chronic inflammatory diseases (Xavier and Podolsky, 2007). The exact nature of susceptibility to either process remains uncertain but many inciting factors seem to be shared by the two diseases (Cho, 2008). Ulcerative colitis and Crohn's disease present with distinctly different patterns of tissue involvement, affect different segments of the gut, and seem to be based on divergent genetic abnormalities. They share genetic variations in the *IL-23 receptor*, *STAT3*, *NKX2–3*, and *IL-12B* genes (Fisher et al., 2008; Franke et al., 2008). The immunological underpinnings of Crohn's disease can be divided into those genetic factors contributing to innate immunity and those involved in adaptive responses. With regard to the former, gene polymorphisms of *NOD2*, *ATG16L1*, and *IRGM* appear to contribute to disease pathogenesis, as these may play important roles in the processing of bacterial-derived factors. Participation of the GH/IGF-I pathway in the pathogenesis of either Crohn's disease or ulcerative colitis has yet to be firmly established, but important alterations in it have been well documented in patients with active inflammatory bowel disease. GH can dampen disease activity and enhance healing in patients with Crohn's disease (Slonim et al., 2000). The GH receptor has been detected in the gut of the rat (Lobie et al., 1990), and thus it may exert direct effects on intestinal inflammation. Han et al. (2005) reported that GH reduces colitis activity in an experimental model using C3H/HeJBir IL-10(−/−) mice, animals exhibiting a propensity for relatively severe forms of large bowel inflammation. GH inhibits apoptosis and enhances the proliferation of crypt epithelial cells and increases mononuclear cell death in the lamina propria. The mechanism underlying the effects of GH on disease activity in this experimental model may involve an increased association between gp130 and SHP-2 and down-regulation of constitutively active STAT3 (Han et al., 2005). The investigators involved in this study also reported, using the same animal model, that blocking the actions of TNF-α with a neutralizing antibody could up-regulate hepatic IGF-I production and GH receptor expression and enhance GH-dependent STAT5 activation (DiFedele et al., 2005). In contrast, TNF-α reduced the abundance of GH receptors and attenuated STAT5 phosphorylation in cultured rat hepatocytes (DiFedele et al., 2005). Thus, it would seem that a complex interplay between pro-inflammatory cytokines and the GH/IGF-I pathway might condition the inflammatory environment of the gut. Moreover, these patterns of molecular cross-talk seem to regulate gut responses to mediators of chronic diseases in these experimental preclinical models. What these findings in animals and cultured cells suggest about human disease remains to be firmly established.

In a study examining 13 patients with Crohn's disease and 7 subjects with ulcerative colitis, IGF-I mRNA was found to be elevated in involved ileum and colon segments from patients with Crohn's disease (Pucilowska et al., 2000). However, the same does not seem to be true for involved colon tissue derived from patients with ulcerative colitis. IGF-I and procollagen αI mRNAs exhibited overlapping distribution in fibrotic submucosa and muscularis propria in Crohn's disease. Moreover, the numbers of IGF-I-expressing mesenchymal cells types was increased in involved regions of bowel in patients with Crohn's disease (Pucilowska et al., 2000). In another study, Zimmermann et al. (2001) reported that IGF-I and IGFBP5 mRNA levels were increased in areas of tissue affected by disease that had been resected from patients with Crohn's disease compared with normal-appearing bowel.

Using immunohistochemistry, El Yafi et al. (2005) detected transmural expression of IGF-IR in inflammatory cells and in smooth muscle cells identified in specimens from patients with ulcerative colitis, diverticuli-

tis, and Crohn's disease (Fig. 11). Infiltrating IGF-IR$^+$ mononuclear cells are far more numerous in the mucosa and submucosa in Crohn's disease. In contrast, the increased frequency of these cells seems to be confined to the mucosa in ulcerative colitis (Fig. 12). In addition, fibroblasts, adipocytes, and hypertrophic nervous plexi displaying IGF-IR seemed peculiar to Crohn's disease (El Yafi et al., 2005). These findings suggest that IGF-IR might play disease-specific roles in the pattern of tissue reactivity and remodeling in inflammatory bowel diseases.

Children with Crohn's disease often exhibit depressed systemic IGF-I levels. The factors underlying this finding remain uncertain. Altered nutrition, such as reduced caloric intake, is frequently encountered in patients with active Crohn's disease and could account for the low IGF-I levels (Kirschner and Sutton, 1986). Twenty-nine children with active disease were examined before and after treatment with either systemic steroids or an elemental diet (Thomas et al., 1993). Median serum IGF-I levels, as determined by radioimmunoassay, were lower among this cohort with disease than those found in matched healthy control subjects and lower than those in children with growth-stunting from other causes. Insulin and IGFBP1 levels were unaffected. IGF-I levels increased after 4 weeks of either dietary intervention or steroid therapy. In another study, children and adolescents with Crohn's disease were assigned to either of two treatment groups (Beattie et al., 1998). The first cohort of 14 subjects received enteral nutrition, whereas the second group of nine underwent surgical intestinal resection (Beattie et al., 1998). In those patients treated with diet modification, CRP fell from pretreatment levels. Median circulating total IGF-I levels, as determined by a formic acid-acetone extraction and radioimmunoassay method, increased rapidly within 2 weeks of treatment initiation, as did serum IGFBP3 levels. Similar trends were observed in the subjects undergoing surgical resections. Serum IGF-I levels increased, whereas CRP concentrations trended downward, but these changes failed to reach statistical significance after 6 months after surgery (Beattie et al., 1998). Studies in adults have demonstrated many of these same effects of bowel inflammation on the IGF-I pathway. In a series of 22 consecutive patients with inflammatory bowel disease, 10 were diagnosed with Crohn's disease, whereas 12 had

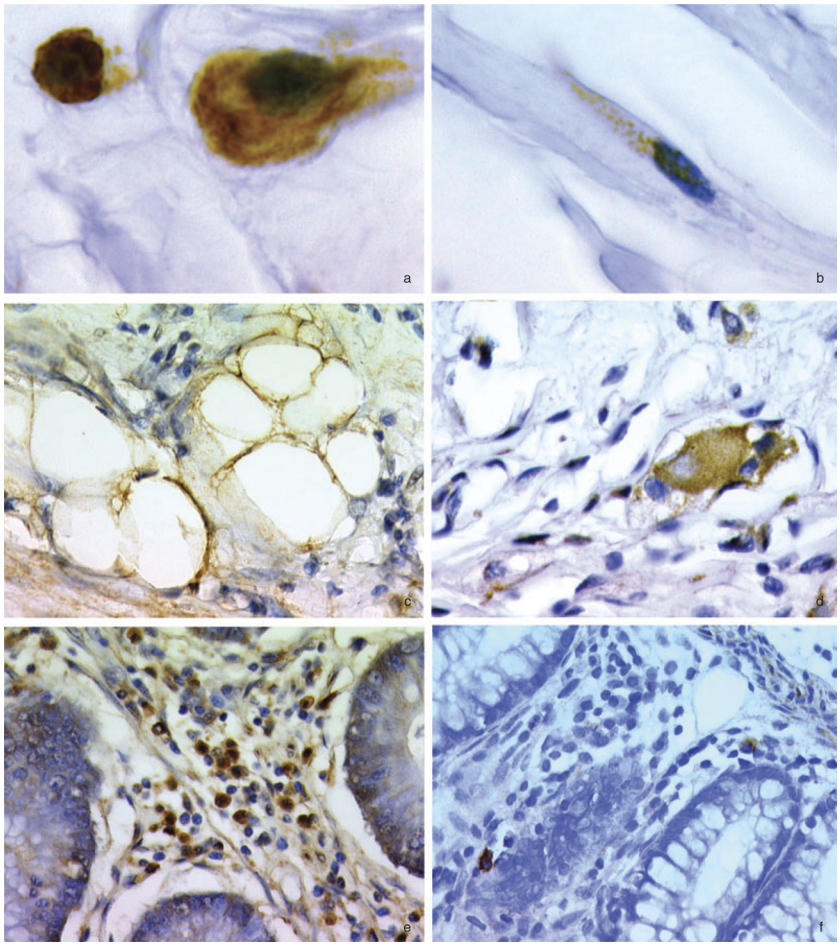

FIG. 11. Evidence of increased abundance of IGF-IR$^+$ cells in Crohn's disease: a, inflammatory cells; b, fibroblastoid cell; c, adipocytes; d, hypertrophied nerve plexus. Note the relative frequencies of IGF-IR$^+$ cells in the lamina propria in uninvolved (e) and disease-involved (f) areas. [Reproduced from El Yafi F, Winkler R, Delvenne P, Boussif N, Belaiche J, and Louis E (2005) Altered expression of type I insulin-like growth factor receptor in Crohn's disease. *Clin Exp Immunol* **139:**526–533. Copyright © 2005 British Society for Immunology. Used with permission.]

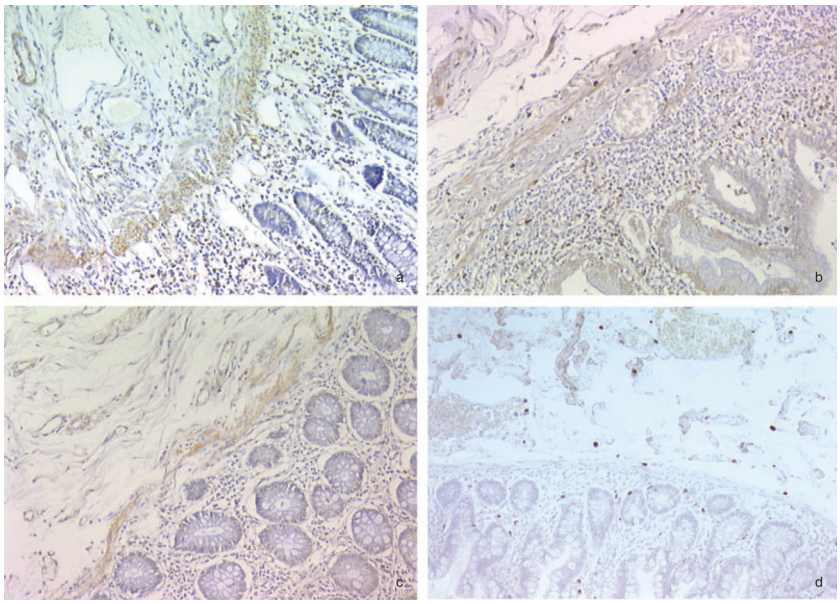

FIG. 12. Immunohistochemical analysis of human bowel. a, increased number of IGF-IR positive inflammatory cells (in brown) in the mucosa and the submucosal in an involved area of Crohn's disease. b, increased number of IGF-IR positive inflammatory cells limited to the mucosa in an involved area of ulcerative colitis. c, no overexpression of IGF-IR in diverticulitis. d, immunohistochemistry with an anti-active caspase 3 antibody: positive inflammatory cells (in red) undergoing apoptosis in an uninvolved area of Crohn's disease. [Reproduced from El Yafi F, Winkler R, Delvenne P, Boussif N, Belaiche J, and Louis E (2005) Altered expression of type I insulin-like growth factor receptor in Crohn's disease. *Clin Exp Immunol* **139:**526–533. Copyright © 2005 British Society for Immunology. Used with permission.].

ulcerative colitis (Katsanos et al., 2001). Serum IGF-I levels were similarly depressed in patients with either disease compared with control subjects. IGFBP3 levels were also lower, whereas the mean serum IL-6 concentration was elevated. In 13 patients with refractory Crohn's disease, total IGF-I and IGFBP3 levels were depressed by 36 and 27%, respectively, before therapy (Eivindson et al., 2007). Both serum markers normalized during treatment with infliximab. In contrast, free IGF-I levels, determined using ultrafiltration, were reduced by 47% pretreatment but failed to normalize. IGFBP2 levels were elevated at baseline by 2.3-fold over controls and fell with therapy. Other markers, such as CRP and serum albumin, also normalized as a consequence of therapy (Eivindson et al., 2007).

Whether the IGF-I/IGF-IR pathway and GH play primary roles in the pathogenesis of inflammatory bowel disease remains uncertain. Although several lines of investigation have identified specific abnormalities in these pathways, especially in Crohn's disease, alterations in many other laboratory-based parameters have also been demonstrated. Some of these are most likely a secondary consequence of the often-severe malnutrition resulting from bowel disfunction. Distinguishing these nonspecific effects from those metabolic and immunologic derangements underlying disease pathogenesis will require further investigation.

3. Rheumatoid Arthritis and Allied Connective Tissue Diseases. IGF-I plays an important role in the regulation of articular connective tissues under normal physiological conditions as well as those associated with disease (Verschure et al., 1996). A hallmark of rheumatoid arthritis (RA), the progressive formation and growth of abnormal tissues, such as panus, suggests that growth factor production and/or activity might play some role in the disease process. Indeed, IGF-I can be detected in synovial fluid from patients with active RA but at levels that do not differ from those found in healthy donors (Matsumoto et al., 1996). Using in situ hybridization, Keyszer et al. (1995) detected transcripts encoding IGF-I and IGF-II in synovial tissues, both from patients with RA and from those with osteoarthritis. The greatest signal intensity was localized to the synovial lining and subsynovial layer. Inflammatory mononuclear infiltrates rarely stained with probes for either growth factor (Keyszer et al., 1995). IGF-I present in synovial fluid may regulate the synthesis of proteoglycans in chondrocytes (Schalkwijk et al., 1989). Neidel et al. (1997) reported elevated levels of serum and synovial fluid IGFBP-2 and IGFBP-3 in patients with RA compared with those found in patients with osteoarthritis. They also found equivalent levels of IGF-I and IGF-II in synovial fluid from control subjects and patients with RA. These stimulated proteoglycan synthesis in cartilage in vitro, activity that could be partially blocked with anti-IGF-I neutralizing monoclonal antibodies (Neidel et al., 1997). Fernihough et al. (1996), on the other hand, reported elevated IGF-I and IGFBP3 levels both in patients with RA and in those with osteoarthritis. Moreover, they found that CRP levels in patients with RA correlated with those of IGF-I, IGF-II, and IGFBP-3 in the synovial fluid. Matsumoto et al. (1996) reported that IGF-I levels as well as those of IGFBP-1, IGFBP-2, IGFBP-3, and

IGFBP-4 were elevated in synovial fluid in patients with RA. This same group of investigators reported that serum IGF-I levels were lower in RA, whereas serum IGFBP-3 levels exceeded those found in control subjects (Matsumoto and Tsurumoto, 2002). Pritchard et al. (2004) found that either IGF-I or IgGs collected from patients with active RA could activate disease-derived synovial fibroblasts to produce IL-16 and RANTES. Both cytokines exhibited biological activity promoting chemotaxis in $CD4^+$ T cells in vitro. In contrast, fibroblasts from patients with osteoarthritis, serving as controls, failed to respond to either the disease-derived IgGs or to IGF-I. It is noteworthy that the effects in RA fibroblasts were mediated through IGF-IR and could also be elicited by GD-IgG from patients with Graves' disease (Fig. 13). Moreover, IgGs from these patients with RA could reciprocally induce IL-16 and RANTES in fibroblasts from donors with Graves' disease (Pritchard et al., 2004). These findings imply that a common disease mechanism might underlie T-cell trafficking in multiple autoimmune disorders, such as Graves' disease and RA. Moreover, the incidence of these diseases is known to cluster in certain families and to cosegregate with greater frequency in susceptible persons. Their findings imply disease specificity in that donors with osteoarthritis, who manifest a chronic inflammatory disease albeit of a nonautoimmune nature, fail to respond to either IGF-I or disease-derived IgG. It is thus possible that the breakdown of peripheral tolerance to IGF-IR results from its overexpression in populations at risk for autoimmune disease.

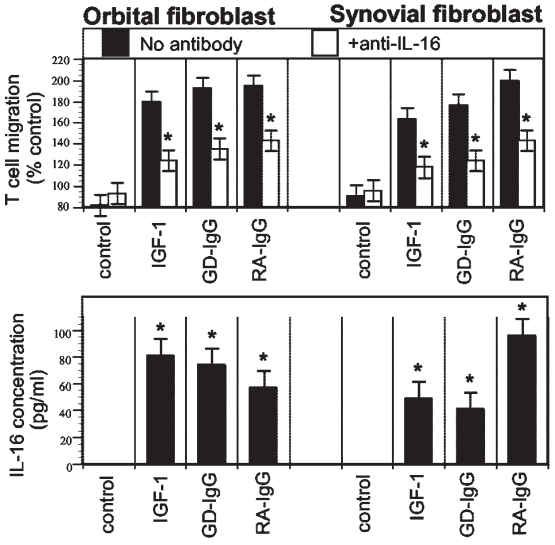

FIG. 13. IL-16-dependent chemoattraction and cytokine expression in GD orbital fibroblasts and RA synovial fibroblasts are induced by both GD-IgG and RA-IgG. Cultures were treated with nothing (control), IGF-I (10 nM), GD-IgG (100 ng/ml), or RA-IgG (100 ng/ml) overnight, and the medium was collected and subjected to T-cell migration assay (top) or enzyme-linked immunosorbent assay (bottom). Data are expressed as the mean ± S.D. of three determinations. *, statistically different migration in the presence of neutralizing Abs (top) or protein production (bottom) at the 5% confidence level. [Reprinted from Pritchard J, Tsui S, Horst N, Cruikshank WW, and Smith TJ (2004) Synovial fibroblasts from patients with rheumatoid arthritis, like fibroblasts from Graves' disease, express high levels of IL-16 when treated with Igs against insulin-like growth factor-1 receptor. *J Immunol* **173:**3564–3569. Copyright © 2004 The American Association of Immunologists, Inc. Used with permission.]

Systemic lupus erythematosus (SLE) is associated with abnormalities of both T- and B-cell function (Kammer et al., 2002; Khan et al., 2003). A hallmark of the disorder is the generation of anti-nuclear antibodies (Tan, 1996). Widespread vascular inflammation in SLE is layered on to organ-specific manifestations and the deposition of immune complexes. Little is currently known about any role that the IGF-I/IGF-IR pathway might play in the pathogenesis of SLE. A single study examining the circulating levels of growth hormone, IGF-I, and somatostatin in age-matched controls and patients with SLE failed to demonstrate any differences (Denko and Malemud, 2004). Although this single report was quite preliminary, its results suggest that abnormally high levels of circulating IGF-I might not be associated with the disease.

Systemic sclerosis represents another vexing chronic process. Interstitial lung disease frequently accompanies systemic sclerosis, but the mechanisms through which pulmonary inflammation and fibrosis occur remain uncertain (Harrison et al., 1991). Although the conventional view embraces inflammation as a precursor of fibroproliferation, recent studies have cast some doubt on the inevitability of that relationship (Krein and Winston, 2002). Most studies have insinuated TGF-β and IGF-I as promoters of fibroblast proliferation and the disordered accumulation of extracellular matrix (Krein and Winston, 2002). Excess collagen is deposited in lung interstitium as a common feature of tissue remodeling. Harrison et al. (1994) have reported that bronchoalveolar lavage fluid from these patients enhances fibroblast proliferation in vitro and that neutralizing antibodies directed against IGF-I can attenuate this effect. Moreover, elevated IGF-I levels were found in this fluid compared with samples from control donors. The findings diverge from an earlier study by Rothe et al. (1988) who reported normal plasma somatomedin C levels in patients with active disease. Reconciling the findings of the two studies suggests that IGF-I may act locally rather than systemically in the disease. Harrison et al. (1994) found that IGF-I levels were elevated only in those patients having abnormal computed tomographic analysis, which was consistent with the disease. Fibroblasts from patients with systemic sclerosis have been shown to respond to growth factors and to produce excessively high levels of glycosaminoglycans (LeRoy et al., 1982; Falanga et al., 1987). Idiopathic pulmonary fibrosis is associated with epithelial cell damage and fibroblast proliferation (Medsger, 1985). In 15 patients with cutaneous disease (morphea), punch biopsies from within lesions were compared with those from normal-appearing skin (Fawzi et al., 2008). Intralesional IGF-I levels

were higher than those found in uninvolved skin. Moreover, these patients exhibited elevated serum IGF-I levels. Positive correlations were observed between the intralesional IGF-I levels and Rodnan scores (Fawzi et al., 2008). The authors concluded that IGF-I antagonists, such as octreotide, deserve consideration as therapeutic strategies. The molecular basis for involvement of IGF-I or IGF-IR in systemic sclerosis has yet to be explored, but the particularly unsatisfactory clinical course associated with the disease suggests that further examination is warranted.

4. Experimental Autoimmune Encephalomyelitis. IGF-I plays an important developmental role in the central nervous system and is synthesized by multiple cell types, including neurons and astrocytes (Bondy, 1991; Lee et al., 1992). Moreover, IGF-I induces oligodendrocyte development (McMorris et al., 1986). Experimental autoimmune encephalomyelitis (EAE) can be induced by immunizing Lewis rats with an emulsion containing guinea pig spinal cord. This animal model resembles that of human multiple sclerosis (Raine, 1984). In rats induced to express EAE, mRNAs encoding IGF-I and glial fibrillary acidic protein were elevated 14 days after disease induction (Liu et al., 1994). Moreover, expression of these transcripts coincided with the appearance of inflammatory infiltrates and demyelination of both white and gray matter (Liu et al., 1994). In contrast, levels of myelin basic protein mRNA were reduced substantially. Experimental animals expressed transiently elevated levels of IGFBP2 mRNA and protein compared with control animals (Liu et al., 1994). Moreover, coexpression of IGF-I and IGFPB2 mRNA was found by in situ hybridization to localize to the same astrocytes. On the other hand, animals treated with exogenous IGF-I (200 μg/day or 1 mg/day) exhibited reduced demyelination, whereas myelin-related protein mRNA levels were induced in oligodendroglial cells (Yao et al., 1995). Larger areas of demyelination were found in spinal cord sections from placebo-treated animals, in addition to substantially more inflammation, and a greater abundance of demyelinated axons compared with those from animals treated with IGF-I (Fig. 14) (Yao et al., 1995). The authors of that study concluded that IGF-I promotes myelination and increases the proliferation of oligodendroglial cells. Their results suggest further that the IGF-I pathway might be manipulated as a strategy for therapeutic intervention in multiple sclerosis and other demyelinating disorders.

VII. Therapeutic Horizons for Autoimmunity: Focusing on Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-I Receptor

Although not yet completely characterized, abnormalities in the IGF-I/IGF-IR pathway associated with autoimmune diseases suggest that its therapeutic interruption might prove beneficial. This would resemble the approach being undertaken in developing cancer therapy. Despite these findings, none of the agents targeting the pathway has been applied to patients with autoimmunity. Common threads of IGF-I dysfunction seem to tie multiple diseases together. One might therefore predict therapeutic benefit in those diseases where IGF-IR is found to be overexpressed, as is the case in Graves' disease, and particularly in those diseases in which receptor activation leads to abnormal responses in im-

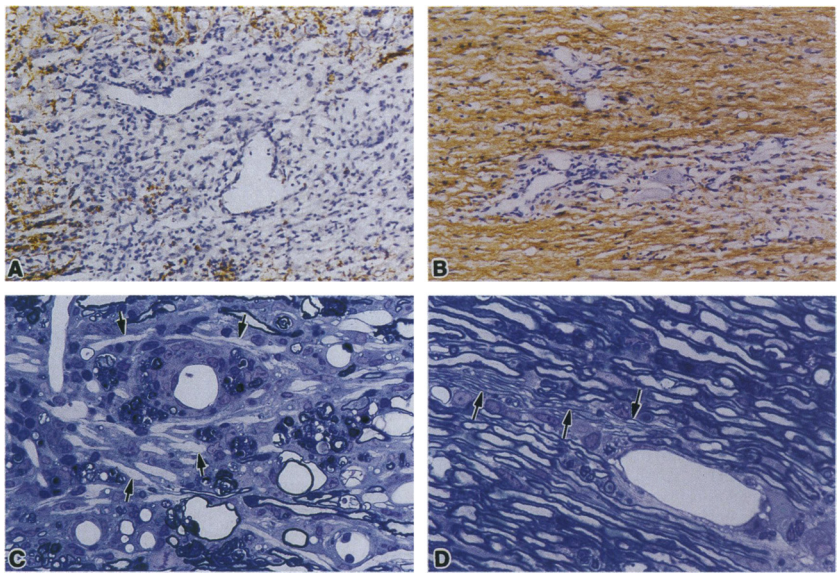

FIG. 14. Thin sections of spinal cord from control rats contain larger demyelinated areas, greater inflammation (A), and a greater abundance of demyelinated axons (arrows in C) than found in IGF-I-treated animals (B and D). Thin, short myelin segments (arrows) surround axons in D. Magnification, 600×. [Reproduced from Yao DL, Liu X, Hudson LD, and Webster HD (1995) Insulin-like growth factor I treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* **92:**6190–6194. Copyright © 1995 United States National Academy of Sciences. Used with permission.]

mune cells. Considering the numerous hurdles that have already been overcome in developing these agents for use in cancer, their application in autoimmunity might represent low-hanging fruit to the pharmaceutical industry.

What can we learn from the substantial efforts expended toward targeting the IGF-I/IGF-IR pathway in neoplastic diseases as we formulate new therapies for autoimmune diseases? Are there molecules currently on the shelf that might be used in several of these diseases in which adequate therapies do not exist? A brief review of the strategies employed to treat cancers might orient discussions surrounding similar targets in chronic inflammatory diseases, such as those with an autoimmune basis. These strategies are nicely reviewed in a recently published article by Kim et al. (2009) focusing on pediatric tumors.

A number of membrane-spanning tyrosine kinase receptors have been implicated in the pathogenesis of several common neoplastic diseases. The fundamental concept of interrupting their function as an important means of treating human illness emerged more than two decades ago (Rondon et al., 2007; Frasca et al., 2008). This notion has been fortified by a greater understanding of how growth factors exert their influence on target tissues and relevant populations of cells. Specifically, these receptors and their downstream signaling cascades are involved in promoting enhanced resistance to apoptosis and increased proliferation of cancer cells. They have thus become rational targets for therapy design (Shawver et al., 2002). Among them, HER1, HER2, platelet-derived growth factor receptor, c-kit, colony-stimulating factor-1 receptor, fibroblast growth factor receptor, and IGF-IR have been identified as therapeutic targets of potential clinical importance. Interest in IGF-IR as an antineoplastic target derives from the discovery that the receptor is overexpressed in many tumor types and that its increased presence and activity may constitute the antiapoptotic and proliferation enhancement associated with several cancers.

IGF-I pathway activation alters cancer cell behavior. It has been implicated in tumor genesis, angiogenesis, metastasis, and mitogenesis in preclinical disease models. IGF-IR may undergo divergent post-translational processing in neoplastic cells. In addition, some tumors exhibit down-regulated levels of IGF-IIR, thereby reducing their capacity to decoy IGF-I and limiting their modulation of its actions (Samani et al., 2007). IGF-IR acts as an oncogene when overexpressed (Kaleko et al., 1990). Because it regulates cell proliferation by mediating mitogenic signals, is necessary for maintaining transformed phenotypes, and protects against apoptosis (Resnicoff and Baserga, 1998), interfering with IGF-IR expression/signaling constitutes a currently attractive approach for drug design. Altering the activity of the IGF-I pathway has been proposed in several diseases, including cancer, diabetes, and atherosclerosis (Sachdev et al., 2006, Clemmons, 2007). It would seem that local concentrations of IGF-I and relative levels of IGF-IR and IGF-IIR determine the activity of this pathway (Butler et al., 1998). Thus, the stoichiometric relationship between these components and the IGFBP family may determine the net impact that IGF-I exerts on tumor cells. Local and circulating concentrations of IGF-I also regulate aspects of tumor cell phenotype, including determining their aggressive behavior. For example, a number of studies have suggested an increased risk of prostate cancer in men with elevated plasma IGF-I levels (Woodson et al., 2003; Oliver et al., 2004).

IGF-I and IGF-II, through their activities mediated by IGF-IR, form important autocrine loops in cancer that promote tumor survival advantage and proliferation. Most of these effects are the consequence of IGF-IR autophosphorylation, the conformational changes favoring its association with four IRS docking proteins, and the activation of MAP kinase and PI3 kinase pathways (Samani et al., 2007). The progressive development of synthetic molecules that can alter the biological impact of growth factors, their cognate receptors, and the signaling cascades mediating their actions now allows testing of the overarching hypothesis that clinical benefit accrues from pathway disruption. Targeting the IGF-I pathway, either alone or in aggregate with others, as a broadly based strategy, seems to hold substantial promise for therapy of several forms of cancer. Drug development targeting IGF-IR can be dissected into small molecule inhibitors and monoclonal antibodies (Imai and Takaoka, 2006). In addition, molecular strategies, such as the application of antisense oligodeoxynucleotides to IGF-IR have been shown to inactivate postreceptor signaling in tumor cells (Salatino et al., 2004). IGF-IR gene silencing can be achieved using small interfering RNA, rendering the target cells more susceptible to cytotoxic agents and radiotherapy (Salisbury and Macaulay, 2003). The enormous advantage of targeting the receptor, rather than its downstream signaling, is the ubiquitous involvement of the latter in critical nonpathological metabolic functions. Initial enthusiasm for IGF-IR-centric drugs was tempered by theoretical concerns about the protein's structural similarities to IR and the potential for physiological promiscuity between the two receptors. Subsequently, the successful development of agents targeting receptors for the epidermal growth factor and vascular endothelial growth factor has refocused the potential of IGF-IR interruption. Agents targeting IGF-IR could emerge as attractive agents for treating autoimmune processes. However, given their generally less dire nature, using these agents in autoimmunity places a greater burden on demonstrating safety compared with their use in malignancy.

VIII. Translational Strategies for Modulation of Insulin-Like Growth Factor-I or Insulin-Like Growth Factor-I Receptor

A. Strategies for Generating Insulin-Like Growth Factor-I Receptor Blocking Antibodies

Biological agents have become a major focus in novel therapeutic development for cancer because of their potential for highly specific molecular and cellular targeting combined with their typically low toxicity compared with small-molecule drugs. The rationale for using multiple agents directed at more than a single target stems from efforts to minimize the emergence of drug resistance and to exploit the effectiveness of combined therapies. Development of antibodies capable of altering the signaling characteristics of IGF-IR began more than 20 years ago, when the receptor's potential importance in cancer pathogenesis became clear. Yamashita et al. (1986) reported that the monoclonal antibody αIR3 could block the effects of IGF-I on growth hormone production in human pituitary adenoma cells. Subsequently, Li et al. (1993) described another monoclonal antibody, designated 1H7, that could block the ligation and activation of IGF-IR. In the years that followed, a number of anti-IGF-IR antibodies have been developed. Doern et al. (2009) recently described an extensive panel of antibodies that can inhibit IGF-IR activation and the downstream events associated with its signaling. They screened and categorized these antibodies and divided them into four groups on the basis of their abilities to block receptor ligation by both IGF-I and IGF-II. They found antibodies that could allosterically block either IGF-I or IGF-II, allosterically block both, or competitively block both IGF-I and IGF-II binding to IGF-IR. Their epitope mapping studies used three separate constructs, including human IGF-IR 1–903, mouse 1–904, and human IGF-IR 1–462, which contains the three N-terminal domains, including L1, the cysteine-rich region, and L2. Using a purified IGF-IR library including 64 mutations, they found that the epitopes recognized by antibodies belonging to all four categories bound overlapping surfaces of the cysteine-rich repeat and L2 domains. The surface epitope map analysis of two monoclonal antibodies, BIIB4 and BIIB5, and a summary of the effect of specific IGF-IR mutations on IGF-I and antibody binding are shown in Fig. 15. BIIB4 blocks IGF-I and IGF-II competitively, whereas BIIB5 blocks both ligands through an allosteric mechanism. Furthermore, the authors found that binding of IGF-I and these antibodies resulted in conformation changes in IGF-IR (Doern et al., 2009). These detailed studies may prove invaluable in dissecting the properties of individual antibodies that render optimal results in subsequent clinical trials.

At least eight different monoclonal antibodies directed at the IGF-IR are currently under development or are being assessed in clinical trials. Each exhibits unique

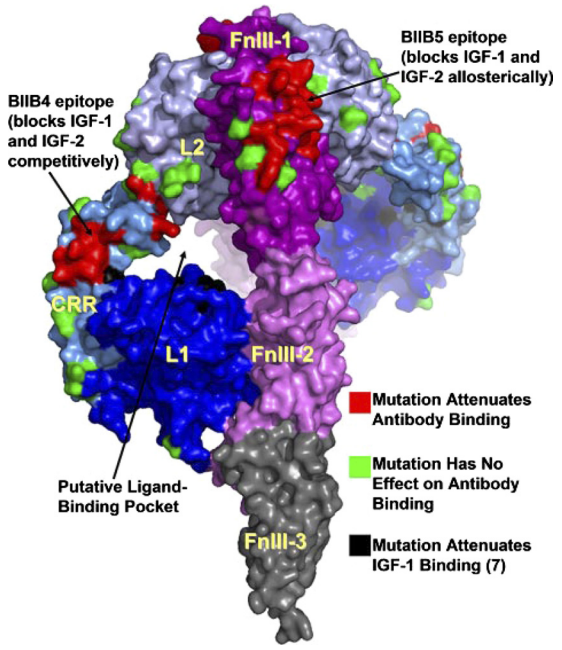

FIG. 15. Results from epitope map analysis of BIIB4 and BIIB5 on the surface of the X-ray crystal structure of the extracellular domains of IR based on homologous positions determined using a sequence alignment of IR and IGF-IR. [Reproduced from Doern A, Cao X, Sereno A, Reyes CL, Altshuler A, Huang F, Hession C, Flavier A, Favis M, Tran H, et al. (2009) Characterization of inhibitory anti-insulin-like growth factor receptor antibodies with different epitope specificity and ligand-blocking properties: implications for mechanism of action in vivo. *J Biol Chem* **284:**10254–10267. Copyright © 2009 The American Society for Biochemistry and Molecular Biology. Used with permission.]

characteristics and has been developed using different strategies. These have been nicely reviewed recently (Feng and Dimitrov, 2008). A spectrum of receptor internalization and blocking activities found among these antibodies should thus put the findings of Doern et al. (2009) into a perspective of clinical efficacy. In brief, figitumumab (CP-751,871) represents a fully human IgG2 exhibiting a binding affinity to IGF-IR of 1.5 nM and an IC_{50} of 0.4 nM for inhibition of IGF-IR autophosphorylation in NIH 3T3/IGF-IR cells (Cohen et al., 2005). This blockade of IGF-I- and IGF-II-provoked phosphorylation was rapid, as was the inhibition of Akt/Pkb activation. CP-751,871 induced a loss of total cellular IGF-IR and the down-regulation of surface receptor as a consequence of internalization. Although it failed to cross-react with IR, this antibody could recognize IGF-IR/IR heterodimeric complexes in MCF7 cells (Cohen et al., 2005). It is noteworthy that this antibody does not exhibit cellular toxicity while inhibiting the proliferation of NIH 3T3/IGF-IR cells in a concentration-dependent manner. This antiproliferative activity was also found in human colon (Colo-205), lung (H460), and breast (MCF7) xenograph models when administered, either as a single agent or in combination with doxorubicin (Adriamycin) or 5-fluorouracil (Cohen et al., 2005). A phase I clinical trial examining CP-751,871 including 24 patients with refractory solid tumors has been completed, and the compound was found to be safe, with

hyperglycemia reported in 4% of patients (Pollak et al., 2007). A phase II study is ongoing and includes 14 subjects with adrenocortical carcinoma and 16 with sarcoma (Feng and Dimitrov, 2008). Nine of the patients with adrenal tumors and three patients with sarcoma exhibited stable disease of a duration greater than 8 weeks (Olmos et al., 2007).

Cixutumumab (IMC-A12) is another fully humanized monoclonal antibody (Burtrum et al., 2003; Rowinsky et al., 2007). It derives from a human naive Fab phage display library. Clones were selected for their ability to block binding of ^{125}I to recombinant IGF-IR. The antibody inhibits the binding of both IGF-I and IGF-II to the receptor on MCF-7 cells but does not alter insulin binding. It also attenuates IGF-I-dependent proliferation of MCF-7, RPMI8226, and BxPC-3 cells in vitro (Burtrum et al., 2003). The latest version of this antibody (A12) binds IGF-IR with an affinity of 40 pM. It blunts IGF-I-dependent IGF-IR phosphorylation and downstream signaling. In human xenograph mouse models, IMC-A12 inhibited the growth of MCF-7, BxPC-3, and Colo205, and it attenuated receptor autophosphorylation, whereas tumor cell apoptosis increased (Burtrum et al., 2003). Multiple phase I trials revealed reduction in absolute lymphocyte counts and hyperglycemia in some subjects. A minority of subjects had stable disease over the course of 4 to 10 months (Higano et al., 2007; Rothenberg et al., 2007; Rowinsky et al., 2007).

BIIB-022 is the product of a human Fab phage display library and represents another fully human antibody with an affinity for IGF-IR of 1.3 nM (Feng and Dimitrov, 2008). It inhibits IGF-I and IGF-II binding and exhibits specificity for IGF-IR in that it fails to bind IR. Early preclinical studies suggest that BIIB-022 will be well tolerated (Hariharan et al., 2007). Determination of its efficacy as an antineoplastic agent awaits the conclusion of ongoing studies. Several characteristics of this antibody suggest that it might prove useful in autoimmune diseases, including its high affinity for the receptor protein and its safety profile.

A humanized monoclonal antibody generated by immunizing mice with IGF-IR, designated h7C10, inhibits the activity of IGF-IR and IGF-IR/IR hybrid proteins (Goetsch et al., 2005). In nude mice harboring either MCF-7 human breast cancer cells or A549 non–small-cell lung cancer xenographs, h7C10 significantly inhibited xenotransplanted tumor growth. The effects were even more dramatic when the antibody was given in combination with another chemotherapeutic agent or with a second antibody targeting EGFR (Goetsch et al., 2005).

The humanized monoclonal antibody R-1507 was generated in human IgG-producing transgenic mice immunized with IGF-IR (Schnitzer et al., 2006). It exhibits antitumor activity in mice harboring 3T3/IGF-IR, NCI-H322M or Colo205 xenotransplants. The drug seems to be well tolerated, although carbohydrate intolerance after oral glucose challenge was detected in some subjects (Rondon et al., 2007). AVE-1642, the human derivative of mouse antibody EM164, has already entered into clinical testing (Feng and Dimitrov, 2008). EM164 blocks IGF-I binding and IGF-I-dependent autophosphorylation of IGF-IR, slows the proliferation of several cancer cell lines in vitro, and results in the regression of BxPC-3 human pancreatic tumors in SCID mice (Maloney et al., 2003). Moreover, it interferes with IGF-IR signaling in CD45$^-$ but not in CD45$^+$ human myeloma cells (Descamps et al., 2006). AVE1642 selectively inhibits the proliferation of CD45$^-$ myeloma cells but enhances bortezomib-induced apoptosis (Descamps et al., 2009). This effect is apparently absent in CD45$^+$ cells and suggests a degree of target cell specificity that might prove attractive in treating autoimmune diseases.

Besides IGF-IR overexpression, some tumors, including those developing from breast epithelium, can form hybrid IGF-IR/IR receptor proteins. Levels of these chimeric proteins can also increase with tumor dedifferentiation (Siddle et al., 1994; Pandini et al., 1999). Specific antibodies exhibit activity selectively against these chimeric receptors (Pandini et al., 1999) and can also downregulate IR levels (Sachdev et al., 2006). Whether chimeric IGF-IR/IR receptors play any role in autoimmune diseases or occur naturally in the immune system is uncertain but must be examined if we are to fully understand the IGF-I pathway in this context.

B. Kinase Inhibitors and Other Nonantibody Targeting of IGF-IR

A summary of the approaches currently considered attractive for altering IGF-IR signaling in disease states has been provided (Fig. 16). Monoclonal antibody development has taken priority by many interested in drug development. Another approach to therapeutically target IGF-IR in cancer involves small-molecule kinase inhibitors with variable degrees of selectivity. Many inhibitory small molecules exhibiting activity against IGF-IR are currently undergoing evaluation for their efficacy against neoplastic diseases. This avenue of discovery has been problematic because of the lack of specificity. Specifically, targeting kinases downstream from IGF-IR with small molecules can effectively attenuate receptor-dependent signaling. However, given the substantial overlap exhibited by many relevant signaling pathways, interrupting these downstream kinases may result in unwanted collateral effects. One strategy for overcoming this commonality has been the utilization of sequence-specific nucleic acid probes, such as antisense and small interfering RNAs. Chemical inhibitor development has focused on the tyrosine kinase domain contained in IGF-IRβ, which can be selectively inhibited with agents such as *cis*-3-[3-(4-methyl-piperazin-l-yl)-cyclobutyl]-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-*a*]pyrazin-8-ylamine (Ji et al., 2007). This recently de-

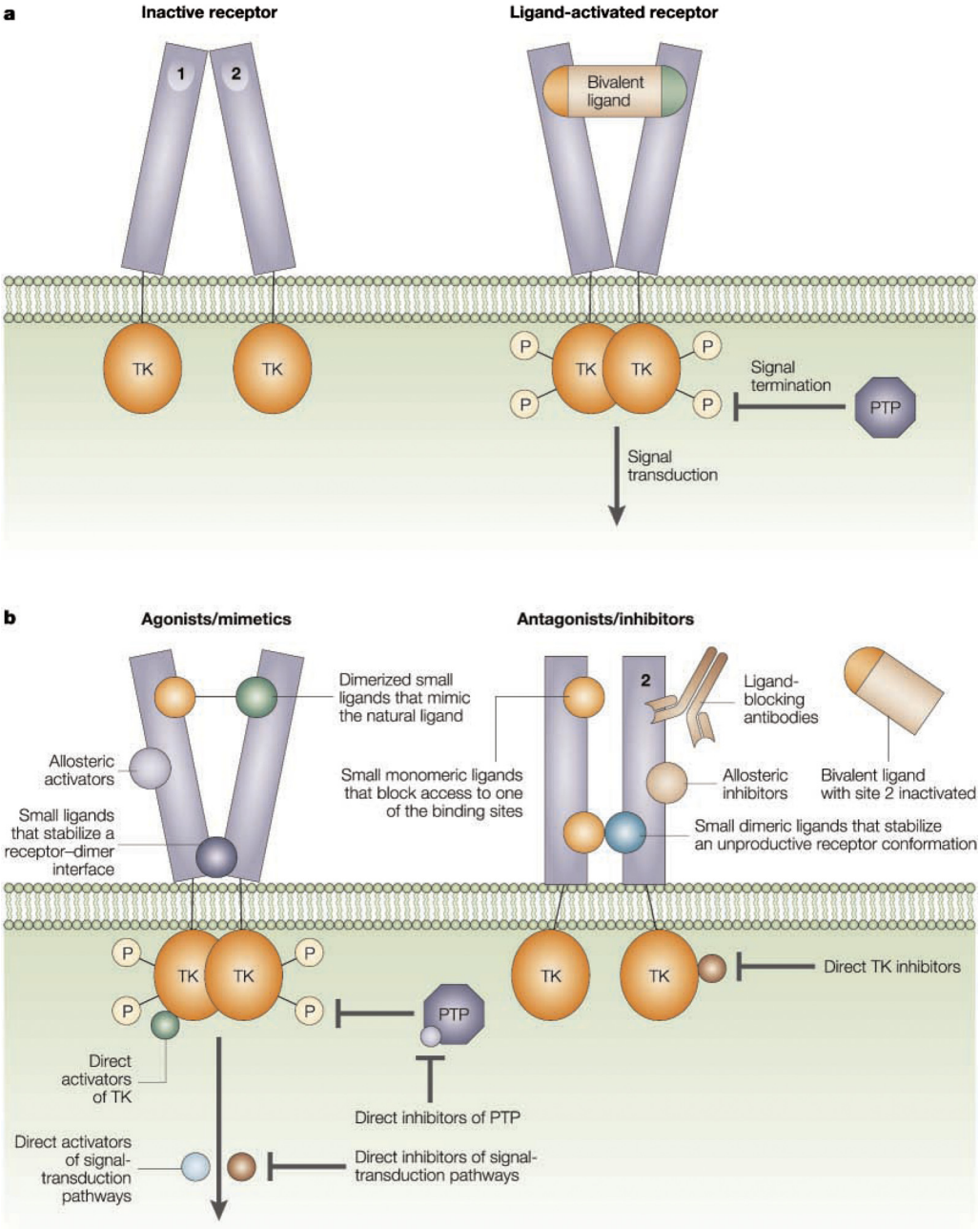

FIG. 16. Strategies for drug discovery with a dimeric or dimerizing receptor tyrosine kinase. a, schematic illustration of the mechanism of receptor tyrosine kinase activation by ligand-induced dimerization. b, schematic illustration of the various possible strategies for the search/design of ligand mimetics and antagonists. PTP, protein tyrosine phosphatase; TK, tyrosine-kinase domain. [Reproduced from De Meyts P and Whittaker J (2002) Structural biology of insulin and IGF1 receptors: implications for drug design. *Nat Rev Drug Discov* **1**:769–783. Copyright © 2002 Nature Publishing Group. Used with permission.]

scribed compound exhibits a high degree of specificity in that 32 other kinases were unaffected or minimally susceptible to its inhibition. In human GEO colon cancer cells, *cis*-3-[3-(4-methyl-piperazin-l-yl)-cyclobutyl]-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-*a*]pyrazin-8-ylamine could block the phosphorylation of Akt and Erk 1/2 and disrupt the IGF-II/IGF-IR autocrine loop (Ji et al., 2007). Nordihydroguaiaretic acid, an inhibitor of the HER2 and IGF-IR tyrosine kinases, blocks the growth of HER2-overexpressing human breast cancer cells (Zavodovskaya et al., 2008). NVP-TAE226 (or TAE226), is a novel dual tyrosine kinase inhibitor of both focal adhesion kinase and IGF-IR that suppresses growth and invasion of glioma cells (Liu et al., 2007). The IGF-IR-specific kinase inhibitor NVP-AEW541 induces apoptosis in acute myeloid leukemia cells exhibiting autocrine IGF-I secretion (Tazzari et al., 2007). 1,3-Disubstituted-imidazo[1,5-*a*]pyrazines might represent important inhibitors of IGF-I action (Mulvihill et al., 2007). Included among them is picropodophyllin, which demonstrates remarkable specificity for IGF-IR and fails to alter tyrosine phosphorylation of the receptors for insulin, fibroblast growth factor, platelet-derived growth factor, or epi-

dermal growth factor (Girnita et al., 2004). This agent induces cell-cycle accumulation and apoptosis in multiple myeloma cells (Strömberg et al., 2006). Cyclolignan picropodophyllin inhibits IGF-IR tyrosine kinase activity through an induction of G_2/M-phase accumulation and apoptosis in multiple myeloma cells (Strömberg et al., 2006). A (1H-benzoimidazol-2-yl)-1H-pyridin-2-one inhibitor of IGF-IR kinase, BMS-536924, exhibits antitumor activity in vivo (Vaira et al., 2007). The selective kinase inhibitor NVP-ADW742 has exhibited substantial antitumor activity in multiple myeloma and solid tumors when administered as monotherapy or in combination with cytotoxic agents (Mitsiades et al., 2004). Each of the compounds exhibits potentially acceptable side-effect profiles that may warrant consideration for use in autoimmune diseases.

In addition to using single-target approaches in modifying IGF-IR activity, combining anti-IGF-IR antibody or IGF-IR kinase inhibitors with a second inhibitor directed at one of the signaling pathways downstream from the receptor might enhance both efficacy and specificity. For instance, monoclonal antibodies against IGF-IR in combination with chemical inhibition of the Raf/MEK/ERK and PI3 kinase/AKT/mTOR pathways can effectively suppress IGF-IR-dependent proliferation in the hematopoietic cell line FDC-P1 rendered IL-3-independent (Bertrand et al., 2006). However, the potential for this approach to attenuate necessary signaling initiated by other receptors makes its safety profile uncertain.

IX. Conclusions

Discovery that IGF-I regulates diverse aspects of T-cell, B-cell, and monocyte function through its interactions with IGF-IR opens several potentially exciting avenues for drug development. Its complex role in immune function suggests that abnormalities in the IGF-I pathway might play some part in the pathogenesis of diseases where immunity is altered. Indeed, recent studies suggest that several autoimmune processes, such as Graves' disease, rheumatoid arthritis, EAE, and inflammatory bowel disease might involve derangements of this pathway. Expression of IGF-I and/or IGF-IR seems to be elevated in these diseases, suggesting similarities with certain forms of cancer. Elevated IGF-IR levels and abnormal IGF-IR-dependent signaling in tumors are currently being exploited as therapeutic targets (Pollak, 2008; Kleinberg et al., 2009). These strategies for drug development in cancer should provide valuable lessons for therapy innovation in autoimmunity. However, the roles of the IGF-I/IGF-IR pathway in chronic autoimmunity are likely to prove very complex. On the one hand, IGF-I seems to protect against pancreatic islet tissue damage in some models of type I diabetes and retards demyelination in animals with EAE. On the other, IGF-IR overexpression may play an important role in the development of Graves' disease and RA, in part by orchestrating the abnormal expansion of pathogenic lymphocytes and their trafficking to sites of inflammation and tissue remodeling. Blocking IGF-IR and the abnormal downstream signaling events it initiates could prove effective therapy for those conditions. One of many remaining questions concerns the apparent absence of increased autoimmunity in transgenic mice overexpressing IGF-IR. If overexpression of that receptor were the only requirement for anti-IGF-IR auto-antibody production, shouldn't we expect these animals to manifest Graves' disease or one of the other allied processes? The answer to this question may lie in the timing and duration of receptor overexpression. For instance, if the higher levels of IGF-IR date back to very early in life, these animals might become peripherally tolerant to the protein. Moreover, the tissue distribution of overexpressed IGF-IR, like any other potential self-antigen, might represent a critical determinant for loss of immune tolerance. Lack of sufficient time for disease development might also explain why these animals have not manifested recognizable disease. Other overarching questions remain, even as we learn more about these diseases and the roles that IGF-I might play in their pathogenesis. Not the least of these uncertainties concerns whether down-regulating IGF-I/IGF-IR function as a therapeutic strategy will ultimately prove sufficiently well tolerated to be used in sublethal diseases. This will be answered ultimately with the successful execution of well designed clinical trials. Nevertheless, hints regarding the suitability of these sorts of interventions already exist. Treatment of autoimmune disease has been dramatically transformed with the emergence of monoclonal antibodies directed at various cytokines (Tincani et al., 2007; Guzman Moreno, 2009), whereas other antibodies have been used for B- and T-lymphocyte depletion (Chatenoud et al., 1994; Hasegawa et al., 2006). Those directed against proinflammatory cytokines such as TNF-α and IL-1 have become mainstays of therapy in many autoimmune diseases, including rheumatoid arthritis, psoriasis, SLE, and multiple sclerosis. Moreover, rituximab has become widely and successfully used as a strategy for depleting CD20 B cells in rheumatoid arthritis, lupus, and a number of other autoimmune diseases (Ahuja et al., 2007; Dörner et al., 2009). Anti-CD3 therapy is under evaluation in type I diabetes (Herold et al., 2002). Success in treating several autoimmune diseases with these antibodies might be considered "proof of principle," because consideration is given to using similar molecules to disrupt IGF-I/IGF-IR signaling. Accumulating additional insight into the abnormal behavior of this pathway in autoimmunity will allow a more thorough assessment of its suitability as a therapeutic target.

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