A number of cytokines that finely regulate immune response have been implicated in the pathogenesis or protection of type 1 diabetes and other autoimmune diseases. It is, therefore, of pivotal importance to examine a family of proteins that serve as signal transducers and activators of transcription (STATs), which regulate the transcription of a variety of cytokines. We report here a defective gene (Stat5b) located on chromosome 11 within a previously mapped T1D susceptibility interval (Idd4) in the nonobese diabetic (NOD) mice. Our sequencing analysis revealed a unique mutation C1462A that results in a leucine to methionine (L327M) in Stat5b of NOD mice. Leu226, the first residue in the DNA binding domain of Stat proteins, is conserved in all identified mammalian STAT proteins. Homology modeling predicted that the mutant Stat5b has a weaker DNA binding, which was confirmed by DNA-protein binding assays. The inapt transcriptional regulation ability of the mutated Stat5b is proved by decreased levels of RNA of Stat5b-regulated genes (IL-2Rβ and Pim1). Consequently, IL-2Rβ and Pim1 proteins were shown by Western blotting to have lower levels in NOD compared with normal B6 mice. These proteins have been implicated in immune regulation, apoptosis, activation-induced cell death, and control of autoimmunity. Therefore, the Stat5b pathway is a key molecular defect in NOD mice.

Studies on the NOD mouse model that spontaneously develops T1D have shown that both B and T cells are necessary for the development of the disease (1–4). Other immune cells such as macrophages and dendritic cells have also been implicated in the disease process (5). Despite extensive studies on this animal model, the underlying molecular mechanisms that lead to the development and progression of T1D still remain elusive. The involvement of cytokines that finely regulate immune and hematopoietic systems in the pathogenesis of diabetes has been an area of intense research. A pathogenic role for interferon-α, interferon-γ, IL-2,1 and IL-10 has been suggested in T1D development in contrast to a protective role for IL-4, IL-6, and tumor necrosis factor α (6–9). Since the link between cytokines and T1D is indisputable, it becomes obligatory to examine the cytokine signaling pathways in relation to the disease. Studies of transcriptional activation by interferons and a variety of cytokines have led to the identification of a family of proteins that serve as signal transducers and activators of transcription (STATs). There are seven identified members in this important family of proteins (Stat1 to Stat4, Stat5a, Stat5b, and Stat6). Upon activation by a large number of cytokines, growth factors, and hormones, STAT proteins are phosphorylated via the Janus kinases, dimerize, translocate to the nucleus, and bind to the promoters of specific target genes (10–13). STAT proteins regulate the expression of a large number of cytokines and their receptors as well as other important molecules involved in immune regulation. In mice, the genes encoding Stat3, Stat5a, and Stat5b map to chromosome 11 (14) within a previously defined Idd4 interval (15). All of the correlative pieces of information led us to explore a plausible connection between T1D and Stat genes on chromosome 11.

This study attempted to use the positional candidate gene approach to test the hypothesis whether functionally chosen candidate genes in the proximity of previously mapped T1D susceptibility intervals are implicated in the disease. We report the identification of a mutation within the DNA binding domain of Stat5b in NOD, which results in diminished DNA binding affinity of Stat5b and reduced expression of downstream genes bearing the Stat5b consensus in their promoter regions.

**MATERIALS AND METHODS**

**Animals and Samples**

Ten-week-old female mice of six strains including C57B1/6J (B6) and NOD/LtJ (NOD), C3H, NZW, BALB/c, and CBA were obtained from our mouse colony at the University of Florida. Total RNA samples were prepared from spleen cells using standard methods. A subset of mice was induced with 0.5 μg (1 μM) of GM-CSF in PBS with 0.1% bovine serum albumin through intraperitoneal injection. After 20 min of induction, the animals were sacrificed, and tissues were immediately snap-frozen in liquid nitrogen and then stored at −80 °C. Sham-treated controls were injected with 500 μl of 0.1% bovine serum albumin in 1 The abbreviations used are: IL, interleukin; STAT, signal transducers and activators of transcription; GM-CSF, granulocytemacrophage colony-stimulating factor; PBS, phosphate-buffered saline; EMSA, electrophoretic mobility shift assay; NOD, nonobese diabetic; FITC, fluorescein isothiocyanate; CREB, cAMP-response-binding protein; OB-R, obese receptor.
Mutant Stat5b Defines a Key Defective Pathway in NOD Mice

0.2-μm filtered PBS (Invitrogen) and sacrificed after 20 min as above. Nuclear and cytoplasmic extracts from spleens were prepared using the method described by Galsgaard et al. (16) with minor modifications. Briefly, tissues were homogenized on ice in hypertonic buffer A (2 mM HEPES, pH 7.9, 10 mM KCl, 1 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 1 mM Na2VO3, 20% glycerol, 1% Triton X-100, 200 μM phenylmethylsulfonyl fluoride, 1 μl/100 ml of phosphatase/inhibitor mixture I, complete protease inhibitor mixture tablet (2 tablets/100 ml). Homogenate was kept for 30 min on ice, and then nuclei were collected by centrifugation at 16,000 rpm at 4 °C for 30 min. Supernatants containing cytosolic protein were transferred to clean tubes and stored at −80 °C for further use. Nuclear protein was extracted from pellets by adding hypertonic buffer B (buffer A with 400 mM NaCl). Each tube was vigorously vortexed for 2 min and then sonicated for 3 s at 18 watts. After centrifugation, 50-μl aliquots of the supernatant (nuclear extract) were made and stored at −80 °C. Protein concentration was measured using the Bio-Rad protein assay.

Electrophoretic Mobility Shift Assays (EMSAs)
Stat5b consensus sequence (5′-TTTCTAGAAATTT-3′) was used for EMSAs, whereas the mutant oligonucleotide 5′-TTTATGTTATTT-3′ was used as a competitor. Complementary oligonucleotides were used to make double strand DNA, which was end-labeled using 170 μCi of [γ-32P]ATP and T4 polynucleotide kinase. Unincorporated isotope was removed using the QIAquick nucleotide removal kit. Labeled oligonucleotides with a count of ~30,000 cpm were used for EMSA. An equal amount of nuclear protein extract (5 μg) was incubated with double strand oligonucleotides (consensus/mutant) at room temperature for 20 min in a binding buffer (13 mM HEPES, pH 7.9, 80 mM NaCl, 8% glycerol, 0.15 mM EDTA, 1 mM dithiothreitol) (17) and in the presence of poly(dI-dC) (2.5 μg/ml). For competition studies, 10-fold excess of unlabelled double-stranded oligonucleotide was added in the preincubation step. For supershift assay, the protein extract was first incubated with 0.8 μg of antibody for 20 min at room temperature. Oligonucleotide was then added, and the reaction continued for another 30 min at room temperature. The anti-Stat3 (SC-482X), anti-Stat5a (SC-1081X), anti-Stat5b (SC-836X), and anti-Nmi (sc-9484X) antibodies are rabbit polyclonal antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). DNA-protein complexes were resolved on a 5% nondenaturing gel containing 5% glycerol and 0.5% TBE. The gels were prerun in 0.5× TBE for 30 min at 150 V 2 μl of loading mix (40% sucrose, 0.25% bromophenol blue, 0.25% xylene cyanol) was added to each sample before loading. The gels were run at 250 V for the first 15 min and then at constant 150 V for about 4 h. The gels were exposed to x-ray films and then kept at −80 °C for at least 48 h.

Immunohistochemistry on Spleen Smears
Spleen Smear Preparation—Experimental and sham-treated control subsets were prepared as detailed above. Spleen was excised, detached of adhering fat, washed in Hank’s balanced salt solution (Invitrogen), and ground between two frosted glass plates in 10 ml of Hank’s balanced salt solution buffer and collected in 15-ml sterile tubes. 1 ml of lysis buffer (Sigma) was added, and the cells were shaken gently on a rocker for 1 min. The cells were transferred in a centrifuge tube and sedimented by gravity for 10 min. The supernatant was aspirated and centrifuged for 7 min at 2000 rpm. The pellet was reconstituted in 1 ml of Hank’s balanced salt solution. The cell density was adjusted to 1 × 107 cells/ml. 50 μl of cells were smeared on polylysine-coated slides. The slides were air-dried in a hood and store at 4 °C in a slide box.

Immunohistochemistry of Spleen Cells—Immunohistochemistry of spleen smears was done as previously described (18). The fixed spleen smears were washed with PBS-T (0.1% Tween 20) (three washings, 10 min each) and neutralized in 100 mM NH4Cl and 0.1% Triton X-100 followed by blocking in 5% normal goat serum for 2 h. The smears were then incubated with primary antibody, viz., anti-Stat5b rabbit polyclonal (sc-835) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) (1:200 dilution for 12–18 h). The smears were washed three times, and the results were averaged. Two peptides were also used in the DNA protein binding assay. These peptides correspond to the DNA binding region (including the mutation L327M of the wild-type Stat5b (DISALVTLTSTFIEKQPPQVLK) and mutant (NOD) Stat5b (DISAMVTSTTFIEKQPPLK) and were purchased from New England Peptide, Inc.
times for 10 min each and subsequently treated with the relevant secondary antibody, *viz.* goat anti-rabbit IgG FITC (BG FITC-2) from Bangalore Genei, India (1:200 dilution) for 60 min. The smears were washed three times for 10 min each and processed for nuclear staining by incubating in a 1:1000 dilution of a 1 mg/ml solution of 4,6-diamidino-2-phenylindole-dihydrochloride hydrate for 15 min. The fluorescence was observed using an excitation wavelength of 450–490 nm with a Nikon B-2A filter for FITC conjugates and a 355–375-nm excitation wavelength with a Nikon UV-1A filter for 4',6-diamidino-2-phenylindole. The results were observed under a Nikon epifluorescence microscope, and the images were captured and documented by the COOL SNAP camera and the Documentation System from Alpha Innotech Corp.

**Northern Blot**

Northern blot was carried out as described previously (19). In brief, 15 μg of total RNA was loaded onto 1.5% agarose gel containing 20% formaldehyde. Electrophoresis was carried out at 150 V for 3 h, and then the gel was blotted to the BrightStar membrane (Ambion) in 20× SSC buffer for overnight. The RNA was cross-linked to the membrane using Crosslinker (Stratagene) according to the manufacturer's instructions. Prehybridization and hybridization were carried out at 42 °C using UltraHyb buffer (Ambion). 3 μg of total RNA was converted to the cDNA by reverse transcription and diluted 1:1 by TE buffer. 2 μl of diluted cDNA was used for PCR amplification of the I-2Rβ and Pim1 genes. The primers for IL-2Rβ are a forward primer (5'-GGATGCCGGAAGGTGATG) and a reverse primer (5'-AGGCAGGTACAGGAAGCTA). The primers for PIM1 are a forward primer (5'-GTGAGCTCGGACTTCTCG) and a reverse primer (5'-GATGGTAGCGAATCCACTCT). The PCR products were loaded onto 2% agarose gel, and then the DNA was recovered from the gel using the Qiaquick gel extraction kit (Qiagen). The purified PCR products were labeled with 32P with a random labeling kit (Stratagene), and then the gel was visualized in a PhosphorImager (Molecular Dynamics). The DNA binding ability of Stat5b was assessed by homology modeling for the wild type and mutant Stat5b. As shown in Fig. 1C, leucine 327 (yellow residue) of the wild type Stat5b establishes contacts with the palindromic DNA binding sequence (TTCCN_GAA) at the T2 positions. The replacement of leucine with methionine (green residue in Fig. 1D) increases the side chain volume and the bulk hydrophobicity around the critical contact point. The local energy values drift from +185 kJ/mol to −33 kJ/mol when methionine replaces leucine. This change would abolish the side chain interdigitation between Leu237 and the thymidine. This introduces a spatial barrier between the Met327 side chain and the thymidine residue (Fig. 1D), which would reduce the ability of methionine to interact with the DNA sequence. Therefore, the mutant Stat5b is predicted to exhibit a weaker DNA binding affinity.

**DNA Binding Affinity of Stat5b**—The DNA binding ability was compared between the wild type (B6) and mutant (NOD) Stat5b using an EMSA. Protein extracts from the cytosolic and nuclear fractions were prepared from splenocytes with and without *in vivo* stimulation with GM-CSF. Western blot with anti-Stat5b antibody showed that the levels of cytosolic fraction, phosphorylated cytosolic fraction, and nuclear Stat5b are comparable in NOD and B6 (Fig. 2A). These results are complemented with immunocytochemical analysis showing similar cytosolic and nuclear Stat5b levels in spleen cells from B6 and NOD mice (Fig. 2B).

**Homology Modeling**

An initial search for templates for homology modeling of Stat5b was carried out using FASTA-CHECK. The search produced two closely similar templates: 1 BFS (the crystal structure of Stat1) and 1BGF (the I-2Rβ protein sequence). The coordinates of these two model templates were used for energy-minimization of the local field energy around the amino acid at position 327 was mapped before and after the introduction of the mutation.

**RESULTS**

**Sequencing of Stat Genes on Chromosome 11**—Three candidate genes within the Idd4 interval on chromosome 11 (Stat3, Stat5a, and Stat5b) were sequenced in NOD and C57BL/6 (B6). Three nucleotide positions in Stat3 and two in Stat5a of both B6 and NOD mice differ from the published sequences (Table I). Since there is no difference between NOD and B6, they are unlikely candidates for diabetes susceptibility factors. Three alterations in Stat5b at positions 433 (G→A), 767 (G→C) and 768 (C→G) are considered as polymorphisms, since they are present in both B6 and NOD animals. Two of the three nucleotide changes are silent mutations. A point mutation (C1462A) in the NOD leads to a substitution of leucine to methionine at position 327 (L327M), the first amino acid of the DNA binding domain of the STAT proteins (Fig. 1, A and B). This mutation was not found in the five control strains (B6, C3H, NZW, BALB/c, and CBA). The Leu237 residue is conserved in all STAT proteins sequenced to date. The potential impact of the L327M mutation on the DNA binding ability of Stat5b was assessed by homology modeling for the wild type and mutant Stat5b. As shown in Fig. 1C, leucine 327 (yellow residue) of the wild type Stat5b establishes contacts with the palindromic DNA binding sequence (TTCCN_GAA) at the T2 positions. The replacement of leucine with methionine (green residue in Fig. 1D) increases the side chain volume and the bulk hydrophobicity around the critical contact point. The local energy values drift from +185 kJ/mol to −33 kJ/mol when methionine replaces leucine. This change would abolish the side chain interdigitation between Leu237 and the thymidine. This introduces a spatial barrier between the Met327 side chain and the thymidine residue (Fig. 1D), which would reduce the ability of methionine to interact with the DNA sequence. Therefore, the mutant Stat5b is predicted to exhibit a weaker DNA binding affinity.

| Gene  | Codon | Published | NOD   | B6   | Effect         |
|-------|-------|-----------|-------|------|---------------|
| Stat3 | 127   | AGG/Lys   | AGG/Glu | AGG/Glu | Amino acid change |
|      | 25    | AGC/Thr   | AGC/Glu | AGC/Glu | Amino acid change |
| Stat5a| 190   | GGA/Gly   | GGC/Gly | GGC/Gly | Silent         |
|      | 527   | AAG/Lys   | AAA/Lys | AAA/Lys | Silent         |
| Stat5b| 372   | CTG/Leu   | CTG/Leu | CTG/Leu | Change in NOD  |
|      | 433   | CGG/Gly   | CGG/Glu | CGG/Glu | Amino acid change |
|      | 767   | CGG/Arg   | CGC/Arg | CGC/Arg | Silent         |
|      | 768   | GTC/Val   | GTC/Val | GTC/Val | Silent         |
B6 form. This does not appear to hold any validity, since in all Western blots the same antibody recognized Stat5b in cytosolic and nuclear extracts with the same affinity, and no differences in the quantities of Stat5b were detectable (Fig. 2A), thus indicating that antigen-antibody binding is not affected by the mutation. Our immunofluorescence data in spleen cells for Stat5b levels show similar quantities in the cytosol and in the nucleus in both B6 and NOD. Once again, this suggests that the antigen-antibody binding is more or less unaffected by the mutation. Additionally, our combined Western and immunofluorescence data suggest similar nuclear export of the protein in both of these models. The antibody sc-835 (Santa Cruz Biotechnology) has been extensively used by others in analyzing Stat5b-specific supershifts (21, 22).

Supershift assays with antibodies against Stat5a and Stat3, which may be associated with Stat5b (23), indicated that the Stat5b complexes do not contain Stat5a (Fig. 3C, lanes 2 and 4) or Stat3 (Fig. 3C, lanes 6 and 8). Together, these results indicate that the weaker Stat5b-specific supershifted complexes in NOD mice were attributable to the impaired DNA binding of the mutant protein. These data are consistent with previous observations that mutations within the DNA-binding region result in Stat proteins that are activated normally but have greatly reduced DNA binding (24–27).

The specificity of the gel shift assay was confirmed using competition with mutated DNA and cold consensus oligonucleotide. The addition of a 10-fold molar excess of cold consensus oligonucleotide caused a complete disruption of the Stat5b-DNA complex in extracts from both B6 and NOD mice, signifying the specificity of the interaction (Fig. 3B, lanes 3 and 6). Further, the use of a hot mutant Stat5b consensus with base replacements at underlined positions 5'-TTA GTT TAA-3' also showed the inability of the mutant oligonucleotide to bind to the Stat5b protein from B6 and NOD nuclear extracts (Fig. 3B, lanes 4 and 7), confirming the specificity of the binding.
sequence-specific transcription factors (28). In order to understand the nature of the nonsupershifted band, we checked whether it can be supershifted using Nmi. The lack of coexistence of Nmi in the protein-DNA complex is also evident from the inability of anti-Nmi to supershift the shifted complex (Fig. 3C, lanes 10 and 12). Thus, we excluded the presence of Stat5a, Stat3, and Nmi in the protein-DNA complex. Centrosomal P4.1-associated protein has recently been shown to specifically interact with Stat5a and Stat5b but not with Stat1 or Stat3 using yeast two-hybrid screen of HBL100 and primary breast adenocarcinoma libraries and is thought to cotranslocate to the nucleus along with activated STAT and augment STAT-mediated transcription (29). We are currently trying to understand the nature of this nonsupershifted band and suspect the involvement of novel yet unidentified Stat5b-interacting partners.

Microbeads-based Assay for DNA Binding—Another possibility for the observed weaker supershift for Stat5b of NOD could be argued as a result of a yet unidentified NOD-specific competitor protein that could change the binding of the Stat5b antibody. To test this unlikely possibility, we evaluated the binding affinity of wild type versus mutant peptides corresponding to the Stat5b binding domain (Fig. 4C). As shown in Fig. 4A (bars 1 and 2), the wild type and mutant Stat5b peptides can bind with Stat5b-consensus oligonucleotide compared with a control oligonucleotide (ATTG-GTTA) (bars 3 and 4). These data suggest that the binding is specific to the Stat5b binding sequence. The inset represents a dot blot of wild type (B6) and mutant (NOD) Stat5b peptide reactivity with Stat5b (sc-835) antibody. None of the controls, comprising Stat5b consensus oligonucleotide plus Cy-labeled secondary antibody (bar 5), Stat5b consensus oligonucleotide plus control antibody (anti-Aire antibody) and Cy-labeled secondary antibody (bar 6), and Stat5b consensus oligonucleotide plus B6 peptide and Cy-labeled secondary antibody (bar 7), showed any detectable binding. The studies also indicated that the binding of the wild type peptide to the Stat5b consensus sequence is ~3.6 times stronger than that of the mutant (NOD) peptide (p < 5.7 × 10−7). This is consistent with the results from EMSA. We also applied the same assay to nuclear protein extracts from NOD and B6 spleens (Fig. 4B). Again, wild type (B6) showed 4 times stronger binding to the Stat5b consensus sequence than mutant (NOD) Stat5b (p < 0.005). Control oligonucleotide did not bind Stat5b (data not shown), showing the specificity of the interactions in the assays.

Expression of Genes Regulated by Stat5b—We examined the impact of the Stat5b mutation on the expression levels of down-
stream genes directly regulated by Stat5b. Two of the genes with potential Stat5b binding sites in their promoter (IL-2Rβ and Pim1) were analyzed as candidate downstream Stat5b effectors. Northern analysis was performed in order to probe alteration in RNA levels to prove the differential transcriptional regulation of normal versus mutated Stat5b. IL-2Rβ mRNA was induced strongly in B6 mice by GM-CSF at both 20-min (p < 10^{-5}) and 2-h (p < 10^{-2}) induction time when compared with NOD mice (Fig. 5A). After 20 min of GM-CSF induction, Pim1 mRNA is induced more strongly in B6 mice compared with NOD mice (p < 0.02) (Fig. 5A). Interestingly, at 2-h induction, the GM-CSF-mediated signal transduction died off for the Pim1 gene, suggesting an early gene regulation.

IL-2Rβ and perforin are shown to have weaker protein expression in splenocytes of Stat5b−/− mice (26). Our immunoblotting assays show weaker protein expression of IL-2Rβ (p < 5.5 × 10^{-6}) and Pim1 (p < 0.001) in NOD splenic extracts compared with B6 splenic extracts (Fig. 5B). These results are consistent with the weaker DNA binding of the mutant Stat5b in NOD followed by lower mRNA levels. Western blot analysis was also performed for Stat3-regulated genes. Cytosolic extracts from the spleen of B6 and NOD mice were probed with

![Fig. 3. DNA binding of Stat5b from B6 and NOD mice assayed using EMSA. A, nuclear extracts from spleens of B6 and NOD mice were incubated with Stat5b-binding consensus sequence TTCN3GAA. Before stimulation with GM-CSF, both B6 and NOD mice showed very weak gel shift (lanes 2 and 6), and Stat5b-specific supershifts were also undetectable (lanes 3 and 7). Stimulation with GM-CSF brought about the appearance of gel-shifted bands in both B6 and NOD animal (lanes 4 and 8). Stat5b-specific supershifts were much stronger in B6 (lane 5) than NOD (lane 9) mice. The Stat5b simple and supershift assays were carried out in six animals from each strain, and the experiment was repeated three times. The histogram on the right represents the mean and S.E. of normalized band intensities of the Stat5b-specific supershifts in B6 and NOD mice. The Stat5b-specific supershifts were 5-fold higher in B6 animals than NOD, and the difference was statistically highly significant (p < 10^{-5}). Lane 1, free label. B, EMSA showing specificity of Stat5b binding. Use of a 10-fold excess of the cold consensus oligonucleotide brought about a total reduction of the gel-shifted band in both B6 (lane 3) and NOD (lane 6) nuclear extracts. A mutant oligonucleotide in gel shift assays did not show shift for B6 (lane 4) or NOD (lane 7). C, antibodies to Stat5a did not bring about supershifts of the shifted bands in B6 (lane 2) or NOD (lane 4). Similarly, anti-Stat3 also failed to introduce supershifts of the shifted complexes in B6 (lane 6) and NOD (lane 8) animals. Nmi, an interacting partner of Stat5b, also did not bring about a supershift in B6 (lane 10) and NOD (lane 12) animals. Lane 1, free label.

![Fig. 3](http://www.jbc.org/)

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antibodies to Bag-1, Mcl-1, Bcl-2, and phosphatidylinositol 3-kinase p85. The levels of these proteins were similar in NOD and B6 mice, indicating that the Stat3-dependent pathways are not impaired in the NOD (Fig. 5C).

DISCUSSION

Our sequencing studies revealed a unique mutation (C1462A) in Stat5b of NOD. The mutation is within the DNA binding domain of the Stat5b protein, and the residue is a conserved amino acid in all the seven members of the STAT gene family as well as in various animal species including mice, rats, sheep, bovines, and humans (Fig. 1). Computer modeling has shown that this substitution of leucine to methionine (L327M) may decrease the binding affinity of the Stat5b protein to DNA. Consequently, we performed a number of studies to experimentally demonstrate the defective function associated with this mutation. Using classical EMSA supershift assay, we showed that the Stat5b protein from NOD has a weaker DNA binding affinity to Stat5b consensus sequence. We have confirmed the weaker binding of NOD Stat5b using a newly developed microbeads-based DNA/protein interaction assay. The differential transcriptional regulation ability of normal and mutated Stat5b is proved by alterations in RNA levels of downstream genes bearing the Stat5b binding motif in their promoter. GM-CSF caused a marked induction of II-2Rβ and
Pim1 mRNA expression in B6 mice, who carry the wild type Stat5b gene. In contrast, GM-CSF induced very weak IL-2Rβ and Pim1 in cytokotic extracts from B6 and NOD spleens without GM-CSF induction and 20 min after GM-CSF induction. Six animals in each strain were analyzed. The mean and S.D. of the signal intensity for each group are presented by the bars on the right, which represent protein levels in GM-CSF-induced B6 and NOD spleen extracts. Student’s t tests were performed to show statistical significance between B6 (gray bars) and NOD (white bars) mice: IL-2Rβ (p < 5 × 10⁻⁶) and Pim1 (p < 0.001).

Many oncogenes such as Myc are capable of inducing both autoimmunity (31). IL-2 and IL-2R knockout mice manifest severe immune system abnormalities characterized by lymphadenopathy, splenomegaly, uncontrolled activation and proliferation of lymphocytes, and T cell infiltration of the bone marrow (32), indicating that IL-2 is a nonredundant determinant of T cell tolerance and autoimmunity. The IL-2 gene is also localized within a type 1 diabetes interval, and a functional polymorphism was observed for the IL-2 molecule in NOD mice (33, 34). Therefore, the decreased level of IL-2Rβ in NOD may contribute to the development of autoimmunity similar to the occurrence in IL-2R⁻/⁻ mice.
cell proliferation and apoptosis (35–38). Pim1 is a serine/threonine kinase, and the protein cooperates specifically with c-Myc in lymphogenesis. Pim1 stimulates c-Myc-mediated apoptosis upstream of caspase-3-like protease. Overexpression of Pim1 can also overcome some of the proliferative defects caused by defective interleukin signaling, supporting a role of Pim1 in cell proliferation (39). Since Myc-induced apoptosis occurs in cells with high Myc protein, the diminished Pim1 expression would lead to a defective c-Myc expression, which may account for lowered apoptosis of cytotoxic T lymphocytes, thus providing them an escape mechanism leading to accumulation of autoreactive T cells in NOD. Indeed, lymphocytes of NOD mice are resistant to several apoptotic stimuli (40, 41). Activated T lymphocytes from NOD showed a markedly increased resistance to induction of apoptosis following deprivation of IL-2 (42). Overexpression of c-Myc counteracts diabetic alterations in transgenic mice through its ability to induce hepatic glucose uptake and utilization and to block the activation of gluconeogenesis and ketogenesis (43).

Leptin and its receptor, obese receptor (OB-R) comprise an important signaling system for regulation of body weight. A point mutation within the OB-R gene of diabetic mice is known to generate a new splice donor, which reduces expression of long form. A short isoform of OB-R is unable to activate the STAT pathway, whereas the long isoforms of OB-R activate Stat5b defective in B6 and NOD mice. A diminished expression of IL-2Rα and Stat5b defective in B6 and NOD mice indicate that the mutation results showing no difference in the Stat5b levels in the cytosol or the nucleus of B6 and NOD levels indicate that the mutation does not affect the nuclear transport of Stat5b in both the models; nor is the sequel of events involved in phosphorylation of Stat5b defective in B6 and NOD mice.

In summary, a mutant Stat5b with weak DNA binding in NOD may exert multiple effects on the development of T1D. The diminished expressions of IL-2Rα, and Pim1 in NOD may explain some of the key malfunctions associated with the disease, including defects in cytokine signaling, T cell selection, and lymphoid cell apoptosis. Furthermore, Stat5b also regulates the expression of many other genes including insulin (16), Tgf-β (43), TGF-β, β-casein (44), Bel-X (47, 48), and insulin-like growth factor. Many of these genes may play critical roles in the pathogenesis of T1D. The discovery of a defective Stat5b pathway provides new avenues to the study of the pathogenesis of T1D, and attempts to rescue the DNA binding ability of mutated Stat5b would be of immense therapeutic importance.

Acknowledgment—We thank Dr. Andrew Muir for insightful comments on the manuscript.

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A Mutant Stat5b with Weaker DNA Binding Affinity Defines a Key Defective Pathway in Nonobese Diabetic Mice
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J. Biol. Chem. 2004, 279:11553-11561.
doi: 10.1074/jbc.M312110200 originally published online December 29, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M312110200

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