In Vitro Correction of JAK3-deficient Severe Combined Immunodeficiency by Retroviral-mediated Gene Transduction

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Summary

Mutations affecting the expression of the Janus family kinase JAK3 were recently shown to be responsible for autosomal recessive severe combined immunodeficiency (SCID). JAK3-deficient patients present with a clinical phenotype virtually indistinguishable from boys affected by X-linked SCID, a disease caused by genetic defects of the common gamma chain (γc) that is a shared component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. The specific interaction of JAK3 and γc represents the biochemical basis for the similarities between these two immunodeficiencies. Both forms of SCID are characterized by recurrent, severe infections leading to death in infancy unless successfully treated by allogeneic bone marrow transplantation. Because of the potentially lethal complications associated with allogeneic bone marrow transplantation and the frequent lack of suitable marrow donors, the development of alternative forms of therapy is highly desirable. To this end, we investigated a retroviral-mediated gene correction approach for JAK3-deficiency. A vector carrying a copy of JAK3 cDNA was constructed and used to transduce B cell lines derived from patients with JAK3-deficient SCID. We demonstrate restoration of JAK3 expression and phosphorylation upon IL-2 and IL-4 stimulation. Furthermore, patients’ cells transduced with JAK3 acquired the ability to proliferate normally in response to IL-2. These data indicate that the biological defects of JAK3-deficient cells can be efficiently corrected in vitro by retroviral-mediated gene transfer, thus providing the basis for future investigation of gene therapy as treatment for JAK3-deficient SCID.

Janus kinases (JAK1, JAK2, TYK2, and the recently identified JAK3) are a family of nonreceptor protein tyrosine kinases involved in intracellular signal transduction mediated by various cytokines, interferons, and growth factors (1, 2). JAK3, unlike the other Janus kinase family members, has an expression pattern restricted to lymphoid and myeloid tissues (3), and appears to play a critical role in cytokine signaling through specific association with the common gamma chain (γc) of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (4–11). JAK3 is activated and phosphorylated upon ligand binding to γc-containing receptors (12–14), an event leading to phosphorylation of several other proteins (including JAK1 and signal transducers and activators of transcription [STATs]), and eventually gene activation (15).

Several lines of evidence have recently underscored the biological relevance of JAK3 and its interaction with γc for development and maturation of lymphoid tissues. First, mutations in γc have been shown to result in X-linked SCID (XSCID) (16, 17), a congenital defect of immunity typically characterized by severe T cell lymphopenia, non-functional B cells, hypoplastic lymphoid organs, and extreme susceptibility to opportunistic infections leading to death early in life (18). Subsequent studies demonstrated that γc mutations responsible for “classical” XSCID phenotype resulted in complete disruption of γc-JAK3 interaction, while a specific point mutation in γc associated with a milder form of combined immunodeficiency was shown to result in a severely diminished, but not completely abrogated, association of JAK3 with γc (11). From this evidence it was postulated that mutations affecting JAK3 function could produce an autosomal form of inherited immunodeficiency.
ficiency resembling the XSCID phenotype. Most recently, in agreement with this prediction, two independent groups identified genetic defects of JAK3 in patients affected by a congenital form of immunodeficiency with cellular and biological characteristics virtually identical to those seen in XSCID, but transmitted as an autosomal recessive trait (19, 20).

After the original reports, additional SCID patients carrying abnormalities of JAK3 expression were identified (Note-

Retroviral Construct and Transduction Procedures. A copy of JAK3 cDNA (3) was subcloned using the NotI and SnaBI sites into the pSAM-EN retroviral cassette by standard molecular biology techniques (25). Amphotropic supernatants were then produced by the "micro ping-pong" technique (26) from PA317 and GP+E-86 cells and used to infect the gibbon-ape leukemia virus (GALV)-based PG13 packaging line. PG13-derived viral particles were then used for subsequent transduction of JAK3-SCID as previously described (27). Transfected and transduced cells were subjected to selection with 0.8-1.0 mg/ml of the neomycin analogue G418 (Geneticin; Life Technologies).

Materials and Methods

Patients and Cell Lines. C.M. and C.A. are JAK3-SCID sibs-

Rapid Determination of IL-2 Activity. IL-2 activity was determined using a liquid scintillation counter.

Results and Discussion

Retroviral-mediated Expression of JAK3. A 3.5-kb fragment containing the full-length JAK3 open reading frame was subcloned into pSAM-EN retroviral cassette to obtain the pGCJ3 vector (Fig. 1). This vector was packaged into GALV envelope expressing retroviral particles using the PG13 packaging line. PG13-derived viral particles were then used to transduce JAK3-deficient BCLs by exposure for 24 h in RPMI medium supplemented with 0.5% FBS and 200 units/ml of IL-2. Transfected cells were sub-

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Figure 1. Schematic representation of the GCJ3 retroviral vector. The 3.5-kb JAK3 cDNA was subcloned into the Moloney murine leukemia virus (MMLV)-based SAM-EN retroviral cassette that contains the internal ribosomal entry sequence from the encephalomyocarditis virus (EMC) and the neomycin resistance gene (neo). The MMLV splice donor site (SD) and the splice acceptor site (SA) in the 3' terminus envelope region (hatched box) are indicated. LTR, long tandem repeat; ψ+, packaging signal.

Figure 2. Analysis of JAK3 protein expression. Lysates of BCLs obtained from normal control (lane 1), JAK3-SCID patient C.M. untransduced (lane 2) and transduced with neo (lane 3) or with JAK3 cDNA (GCJ3, lane 4), and JAK3-SCID patient C.A. untransduced (lane 5) or transduced with JAK3 cDNA (lane 6) were subjected to SDS-PAGE and then electro-transferred to nylon filter. The membrane was immunoblotted (IB) with α-JAK3 (top), then stripped and rebotted with α-JAK1 (bottom) to verify equal loading.

Figure 3. Analysis of JAK3 phosphorylation. Normal control (lanes 1–3), untransduced (4–6) and GCJ3-transduced (lanes 7–9) JAK3-SCID BCLs were stimulated with the indicated cytokine for 15 min at 37°C, lysed, and immunoprecipitated (IP) with α-JAK3. Complexes were resolved by SDS-PAGE and detected by immunoblotting (IB) with α-PY. Membrane was stripped and the presence of JAK3 in immunocomplexes was verified by blotting with α-JAK3.

Phosphorylation of JAK3 (Fig. 3, top). Normal donor BCLs (lanes 1–3), untransduced (lanes 4–6), and JAK3-transduced (lanes 7–9) JAK3-SCID patient cells were left unstimulated (lanes 1, 4, and 7) or stimulated with IL-2 (lanes 2, 5, and 8), or IL-4 (lanes 3, 6, and 9). Lysates were immunoprecipitated with α-JAK3 and immunocomplexes probed with α-PY. We observed that the transduced JAK3 protein was properly phosphorylated in response to both cytokines, thus demonstrating that the signal generated by IL-2 and IL-4 ligand binding was appropriately transduced to the newly expressed JAK3 protein. The filter was then stripped and reprobed with α-JAK3 to confirm the presence of JAK3 protein in the immunoprecipitates (Fig. 3, bottom). As expected from the absence of JAK3 in whole cell lysate (Fig. 2, lane 2), no JAK3 protein was immunodetected in the JAK3-SCID cells before gene transfer (lanes 4–6). Taken together these data further suggest that...
the transduced gene product was capable of functionally associating with $\gamma_c$, thus restoring the JAK3-$\gamma_c$ interaction, a critical component of signaling through all $\gamma_c$-containing receptors (11).

**IL-2-mediated Cell Proliferation.** Previous studies (29, 30) have indicated a primary role for JAK3 in cell proliferative response to IL-2, thus suggesting the involvement of JAK3 in IL-2-induced cell cycle progression mechanisms. The results of these studies would predict a defective proliferative response to IL-2 in cells from JAK3-deficient patients. To determine whether JAK3-SCID BCLs showed reduced proliferation in response to IL-2 and whether JAK3 gene transfer and expression could restore a normal response, we assayed IL-2-induced cell proliferation before and after gene transduction. We previously determined appropriate culture conditions that allowed us to demonstrate a specific proliferative response of BCLs to IL-2 (27). Using these same conditions, we could detect substantially reduced cell proliferation of JAK3-SCID BCLs to IL-2 when compared to normal donor BCLs. Moreover, we demonstrated normalization of IL-2-mediated cell growth after JAK3 gene transfer (Fig. 4), but not after transduction with the neo control retroviral vector. It should be noted that EBV-immortalized B cell lines are not IL-2 growth dependent, and therefore the effect of IL-2 on cell growth is not as dramatic as it is for primary cells or factor-dependent lines. Nonetheless, lack of proliferation to IL-2 in JAK3-SCID cells confirms the importance of JAK3 for cell cycle progression induced by this cytokine. Furthermore, the restoration of an appropriate proliferative response upon gene correction demonstrates that the exogenously expressed JAK3 protein allowed for proper transduction of the IL-2 signal from receptor to nucleus, leading to transcriptional activation of specific genes responsible for cell growth. Moreover, the generation of gene-corrected JAK3-SCID cells will be of considerable importance in further analysis of general pathways of cytokine signaling where JAK3 kinase is involved (Oakes, S.A., F. Candotti, J.A. Johnston, J.J. Ryan, N. Taylor, L. Henninghausen, L.D. Notarangelo, W.E. Paul, R.M. Blaese, and J.J. O'Shea, manuscript in preparation).

Severe combined immunodeficiencies are a heterogeneous group of inherited disorders of immunity characterized by failure to thrive, severe infections, and combined lack of T and B cell functions. Infectious episodes are usually recurrent, life-threatening, and lead to death in early life unless affected patients undergo reconstitution of a normal immune system by allogeneic bone marrow transplantation (31). Transplantation procedures from HLA-identical siblings usually result in full immune reconstitution of the recipients with minimal risks related to GVHD. In contrast, transplantation from haplo-identical donors or MHC-matched unrelated individuals is characterized by a higher risk of severe GVHD and graft failure (21, 32). Gene therapy, therefore, could represent a beneficial alternative form of treatment for those patients lacking a suitable marrow donor.

The recent identification of defects in JAK3 protein expression as the cause of an autosomal recessive form of SCID (19, 20) has on the one hand opened the way for a better understanding of the mechanisms involved in lymphocyte development and differentiation, and on the other provided the basis for investigating gene-based therapeutic approaches. We demonstrate here that retroviral-mediated gene transduction could effectively correct several biological defects in B cell lines from JAK3-SCID patients. Because of the lack of mature circulating T cells, these patients are not eligible for a T lymphocyte-directed gene transfer approach as previously used for adenosine deaminase-deficient SCID (33); consequently, corrective gene transfer for JAK3-deficient patients will require targeting of the lympho-hematopoietic stem cell. Retroviral vectors have been demonstrated capable of safely transferring and expressing exogenous genes into hematopoietic progenitors in animal models (34–36) and humans (37–39). It is conceivable, therefore, that the genetic correction and reinforcement of autologous JAK3-deficient lympho-hematopoietic stem cells could provide a means to reconstitute the immune system of JAK3-SCID patients, avoiding the risks of GVHD.

Further studies are necessary to assess whether the "ectopic" expression of retrovirally transduced JAK3 in a stem cell could be deleterious to the normal development of non-lymphoid hematopoietic lineages before a clinical gene therapy protocol for JAK3-SCID could be proposed. As part of pre-clinical investigations, the transfer of the JAK3 gene into the hematopoietic system of animal models would provide important insight into the biological effects of the exogenous expression of JAK3 protein. The recent generation of mice with a targeted deletion of the JAK3 gene (40–42) constitutes an important model where these critical issues can be conveniently addressed.
Furthermore, the definition of the JAK3 genomic structure (Riedy, M.C., W. Modi, A.S. Dutra, T.B. Blake, B.K. Lal, J. Davis, A. Bosse, J.J. O’Shea, and T.A. Johnston, manuscript submitted for publication) and the future identification of its regulatory region will provide the possibility of constructing gene transfer vectors containing the natural promoter sequences and leading to physiologically controlled JAK3 expression.

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