Brief Definitive Report

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 (γ_c) 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 γ_c 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 (γ_c) 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 γ_c-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 γ_c for development and maturation of lymphoid tissues. First, mutations in γ_c have been shown to result in X-linked SCID (XSCID) (16, 17), a congenital defect of immunity typically characterized by severe T cell lymphopenia, nonfunctional B cells, hypoplastic lymphoid organs, and extreme susceptibility to opportunistic infections leading to death early in life (18). Subsequent studies demonstrated that γ_c mutations responsible for "classical" XSCID phenotype resulted in complete disruption of γ_c-JAK3 interaction, while a specific point mutation in γ_c associated with a milder form of combined immunodeficiency was shown to result in a severely diminished, but not completely abrogated, association of JAK3 with γ_c (11). From this evidence it was postulated that mutations affecting JAK3 function could produce an autosomal form of inherited immunode-

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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 (Notarangelo, L.D., unpublished observations), defining JAK3-deficient SCID (JAK3$^-$SCID) as a separate nosological entity. On the other hand, because of the clinical similarities existing between JAK3$^-$SCID and XSCID (19, 20), the medical management is identical for both forms of immunodeficiency, and allogeneic bone marrow transplantation (BMT) is currently the only curative treatment available. BMT, however, is an intensive procedure potentially associated with severe complications such as graft versus host reactions (GVHD) (21), and is not an available option for many patients because of lack of suitable marrow donors. For these reasons, the investigation of alternative therapeutic strategies is needed, and the development of gene-based therapies would be beneficial for those cases where traditional treatment is not available.

To investigate a gene therapy approach for JAK3$^-$SCID, we constructed a retroviral vector containing the normal JAK3 cDNA sequence and used it to reconstitute the expression of JAK3 protein in B cell lines from JAK3$^-$SCID patients. We show that the transgenic JAK3 was expressed at levels comparable to those found in B lymphoblastoid cells from normal donors. Further, we show that the newly expressed JAK3 kinase was phosphorylated upon IL-2 and IL-4 stimulation, and that IL-2-mediated cell proliferation was restored to nearly normal levels in JAK3-transduced cells. This study demonstrates that biological functions affected in JAK3-deficient cells can be corrected in vitro by retroviral-mediated gene transfer and suggests the potential application of gene transfer as therapy for JAK3$^-$SCID.

Materials and Methods

Patients and Cell Lines. C.M. and C.A. are JAK3$^-$SCID siblings from consanguineous parents whose mutation has been previously reported (19). Peripheral blood was obtained upon parental informed consent from both patients and normal donors and B cell lines (BCLs) were established by standard Epstein-Barr virus immortalization. Cells were maintained in RPMI 1640 (GIBCO BRL, Gaithersburg, MD), and cultured at 37°C, 5% CO_2. PA317 (22), PG13 (23), and GP+E-86 (24) retrovirus-packaging lines were cultured in Dulbecco's modified Eagle's minimum essential medium (DME; Biofluids, Rockville, MD), at 37°C, 10% CO_2. All tissue culture media were supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 2 mM L-glutamine (GIBCO BRL), and 50 μg/ml gentamycin (Bio-Whittaker, Walkersville, MD).

Retroviral Construct and Transduction Procedures. A copy of JAK3 cDNA (3) was subcloned using the Not*I* and Sna*BI* sites into the pSAM-EN retroviral cassette by standard molecular biology techniques (25). Amphotropic supernatants were 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).

Western Protein Analysis. Cells were lysed in buffer containing 300 mM NaCl, 50 mM Tris-HCl, 2 mM EDTA, 0.5% Triton X-100, 2.5 μM *p*-nitrophenyl *p'*-guanidino-benzoate, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. Lysates (100 μg of protein) were centrifuged at 12,000 *g*, boiled, subjected to 8% SDS-PAGE, and electro-transferred onto nylon membranes (Immobilon-P; Millipore, Bedford, MA). Membranes were incubated with a rabbit polyclonal Ab directed against the COOH terminus of JAK3 (α-JAK3) (3) or a mouse monoclonal Ab specific for JAK1 (α-JAK1; Transduction Laboratories, Lexington, KY) following previously reported procedures (12).

Immunoprecipitation. Cells (5–10 × 10^7) were cultured in RPMI 1640 without FBS for 4 h, stimulated with IL-2 (1,000 U/ml) or IL-4 (1,000 U/ml) for 15 min, and then lysed in the above defined lysis buffer supplemented with 200 μM sodium orthovanadate. After centrifugation, postnuclear supernatants were immunoprecipitated with α-JAK3, washed, boiled, and resolved using SDS-PAGE electrophoresis. Immunoprecipitated complexes were immunoblotted with anti-phosphotyrosine mouse monoclonal Ab (α-PY, 4G10; Upstate Biotechnology Inc., Lake Placid, NY) or α-JAK3 as described (12). Detection was then performed by enhanced chemiluminescence (ECL; Amersham Corp., Arlington Heights, IL).

Proliferative Response to IL-2. Cells were incubated in RPMI 1640 supplemented with 0.5% FBS for 24 h. Cells (2 × 10^4/well) were then plated onto 96-well plates and incubated for 24 h in RPMI with or without 2.5–5 × 10^{-7} M IL-2. Tritiated thymidine ([^{3}H]-Tdr, 1 μCi/well; NEN-Dupont, Boston, MA) was then added to microcultures and incubated for an additional 6 h. Cells were harvested with an automated cell harvester (Wallac, Uppsala, Sweden) and transferred to filters. DNA-incorporated radioactivity was determined using a liquid scintillation counter. Results are given as average of six replicates. Stimulation index was calculated as the ratio of [^{3}H]-Tdr incorporated in the presence of RPMI medium supplemented with IL-2 divided by that incorporated in RPMI medium without IL-2 supplementation.

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 cell line since this envelope protein results in higher rates of retroviral transduction of lymphoid cells (28). Supernatants containing retroviral particles were then used to transduce JAK3-deficient BCLs by exposure for 24 h in presence of 10 μg/ml protamine. After G418 selection, the transduced cells were assessed for JAK3 protein expression. Fig. 2 (*upper panel*) shows the results of Western analysis on whole cell lysates obtained from normal BCLs (lane *1*), patients C.M. and C.A. before (lanes *2* and *5*, respectively) and after transduction with GCJ3 (lanes *4* and *6*, respec-

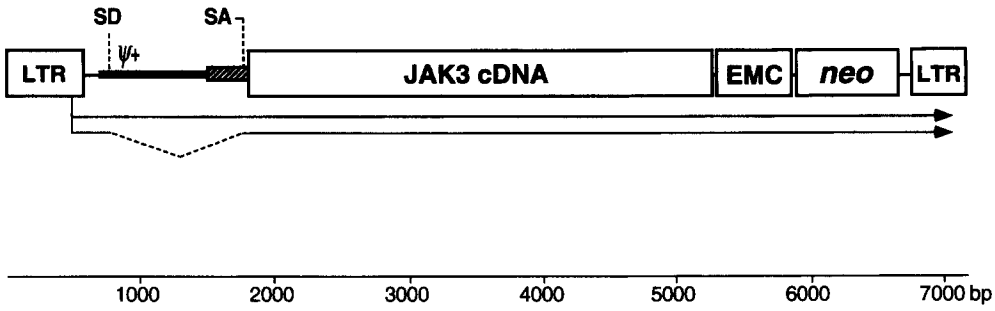

Figure 1. Schematic representation of the GCJ3 retroviral vector. The 3.5-kb JAK3 cDNA was subcloned into the Moloney murine leukemia virus (MMLV)-based SAM-EN retroviral cassette that contains the internal ribosomal entry sequence from the encephalomyocarditis virus (*EMC*) and the neomycin resistance 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.

tively). Lane *3* represents cell lysate from patient C.M. after transduction with SAM-EN control retroviral vector containing only the neomycin resistance gene (*neo*). GCJ3-transduced cells were shown to newly express JAK3 protein of expected molecular weight and at levels comparable to controls. Cells transduced with the control vector carrying only *neo* did not show increased expression of JAK3. The filter was stripped and reprobed with α-JAK1 (*lower panel*) to verify equal loading in all lanes. These data demonstrate that in vitro gene delivery using retroviral vectors is an efficient method to restore high levels of expression of JAK3 protein in cells from JAK3$^-$SCID patients.

JAK3 Phosphorylation Analysis. To examine whether the expression of JAK3 would restore cytokine responsiveness in transduced cells, we analysed IL-2- and IL-4-induced 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

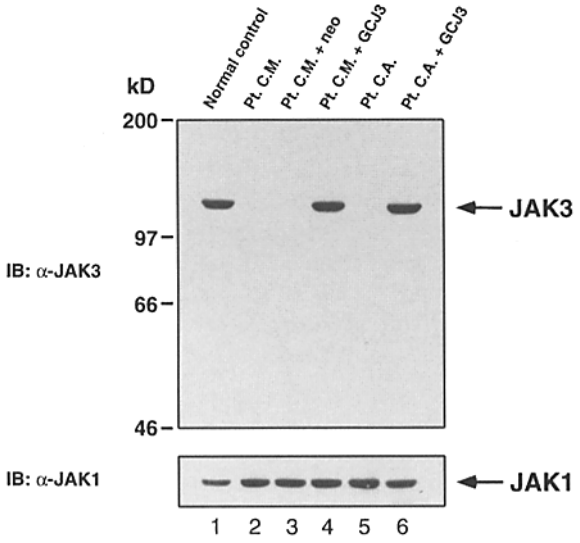

Figure 2. Analysis of JAK3 protein expression. Lysates of BCLs obtained from normal control (lane *1*), JAK3$^-$SCID patient C.M. untransduced (lane *2*) and transduced with *neo* (lane *3*) or with JAK3 cDNA (GCJ3, lane *4*), and JAK3$^-$SCID patient C.A. untransduced (lane *5*) or transduced with JAK3 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 reblotted with α-JAK1 (*bottom*) to verify equal loading.

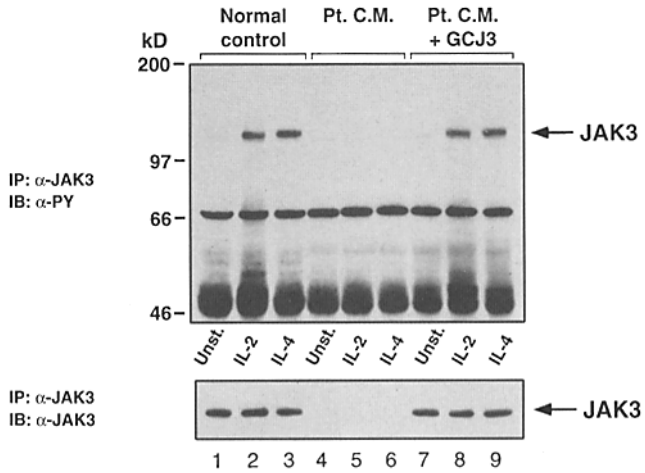

Figure 3. Analysis of JAK3 phosphorylation. Normal control (lanes *1–3*), untransduced (*4–6*) and GCJ3-transduced (lanes *7–9*) JAK3$^-$SCID BCLs were stimulated with the indicated cytokine for 15 min at 37°C, lysed, and immunoprecipitated (*IP*) with α-JAK3. Complexes were resolved by SDS-PAGE and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

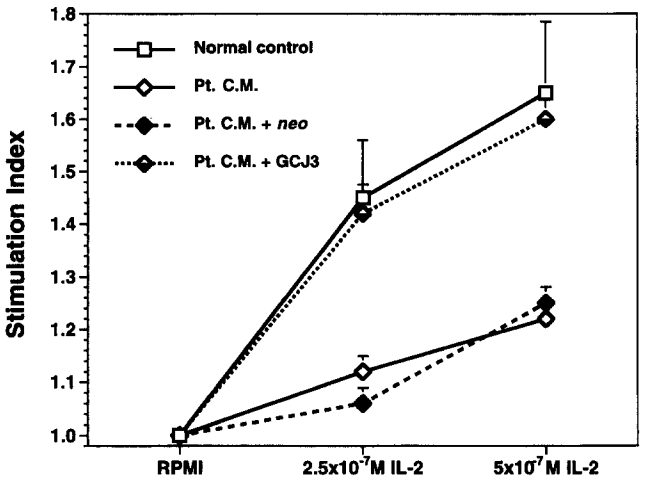

Figure 4. Effects of IL-2 on cell proliferation. BCLs were stimulated for 30 h with IL-2 and proliferation, determined by DNA incorporation of tritiated thymidine, indicated as stimulation index (ratio between average incorporated [^{3}H]-Tdr in the presence of IL-2 to that in the absence of IL-2). Cpm values in RPMI medium without IL-2 were as follows (average ± SD): normal BCLs, 2,748 ± 207; patient C.M., 1,239 ± 91; patient C.M. transduced with *neo*, 960 ± 72; patient C.M. transduced with GCJ3, 1,087 ± 83.

the transduced gene product was capable of functionally associating with γ_c, thus restoring the JAK3-γ_c interaction, a critical component of signaling through all γ_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 reinfusion 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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References

1. Taniguchi, T. 1995. Cytokine signaling through nonreceptor protein tyrosine kinases. *Science (Wash. DC).* 268:251–255.
2. Ihle, J.N. 1995. Cytokine receptor signalling. *Nature (Lond.).* 377:591–594.
3. Kawamura, M., D.W. McVicar, J.A. Johnston, T.B. Blake, Y.Q. Chen, B.K. Lal, A.R. Lloyd, D.J. Kelvin, J.E. Staples, J.R. Ortaldo, and J.J. O'Shea. 1994. Molecular cloning of L-JAK, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. *Proc. Natl. Acad. Sci. USA.* 91:6374–6378.
4. Takeshita, T., H. Asao, K. Ohtani, N. Ishii, S. Kumaki, N. Tanaka, H. Munakata, M. Nakamura, and K. Sugamura. 1992. Cloning of the gamma chain of the human IL-2 receptor. *Science (Wash. DC).* 257:379–382.
5. Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K. Arai, K. Sugamura, M. Matsuoka, T. Takeshita, N. Ishii, M. Nakamura, T. Ohkubo, and K. Sugamura. 1993. Sharing of the interleukin-2 (IL-2) receptor gamma chain between receptors for IL-2 and IL-4. *Science (Wash. DC).* 262: 1874–1877.
6. Russell, S.M., A.D. Keegan, N. Harada, Y. Nakamura, M. Noguchi, P. Leland, M.C. Friedmann, A. Miyajima, R.K. Puri, W.E. Paul, and W.J. Leonard. 1993. Interleukin-2 receptor gamma chain: a functional component of the interleukin-4 receptor. *Science (Wash. DC).* 262:1880–1883.
7. Noguchi, M., Y. Nakamura, S.M. Russell, S.F. Ziegler, M. Tsang, X. Cao, and W.J. Leonard. 1993. Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. *Science (Wash. DC).* 262:1877–1880.
8. Kondo, M., T. Takeshita, M. Higuchi, M. Nakamura, T. Sudo, S. Nishikawa, and K. Sugamura. 1994. Functional participation of the IL-2 receptor gamma chain in IL-7 receptor complexes. *Science (Wash. DC).* 263:1453–1454.
9. Kimura, Y., T. Takeshita, M. Kondo, N. Ishii, M. Nakamura, J. Van Snick, and K. Sugamura. 1995. Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. *Int. Immunol.* 7:115–120.
10. Giri, J.G., M. Ahdieh, J. Eisenman, K. Shanebeck, K. Grabstein, S. Kumaki, A. Namen, L.S. Park, D. Cosman, and D. Anderson. 1994. Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:2822–2830.
11. Russell, S.M., J.A. Johnston, M. Noguchi, M. Kawamura, C.M. Bacon, M. Friedmann, M. Berg, D.W. McVicar, B.A. Witthuhn, O. Silvennoinen et al. 1994. Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. *Science (Wash. DC).* 266:1042–1045.
12. Johnston, J.A., M. Kawamura, R.A. Kirken, Y.Q. Chen, T.B. Blake, K. Shibuya, J.R. Ortaldo, D.W. McVicar, and J.J. O'Shea. 1994. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature (Lond.).* 370: 151–153.
13. Witthuhn, B.A., O. Silvennoinen, O. Miura, K.S. Lai, C. Cwik, E.T. Liu, and J.N. Ihle. 1994. Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature (Lond.).* 370:153–157.
14. Johnston, J.A., C.M. Bacon, D.S. Finbloom, R.C. Rees, D. Kaplan, K. Shibuya, J.R. Ortaldo, S. Gupta, Y.Q. Chen, J.D. Giri, and J.J. O'Shea. 1995. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. *Proc. Natl. Acad. Sci. USA.* 92:8705–8709.
15. Ihle, J.N. 1996. STATs: signal transducers and activators of transcription. *Cell.* 84:331–334.
16. Noguchi, M., H. Yi, H.M. Rosenblatt, A.H. Filipovich, S. Adelstein, W.S. Modi, O.W. McBride, and W.J. Leonard. 1993. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell.* 73:147–157.
17. Puck, J.M., S.M. Deschenes, J.C. Porter, A.S. Dutra, C.J. Brown, H.F. Willard, and P.S. Henthorn. 1993. The interleukin-2 receptor gamma chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. *Hum. Mol. Genet.* 2:1099–1104.
18. Conley, M.E. 1991. X-linked severe combined immunodeficiency. *Clin. Immunol. Immunopathol.* 61:S94–S99.
19. Macchi, P., A. Villa, S. Giliani, M.G. Sacco, A. Frattini, F. Porta, A.G. Ugazio, J.A. Johnston, F. Candotti, J.J. O'Shea et al. 1995. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature (Lond.).* 377:65–68.
20. Russell, S.M., N. Tayebi, H. Nakajima, M.C. Riedy, J.L. Roberts, M.J. Aman, T.S. Migone, M. Noguchi, M.L. Markert, R.H. Buckley et al. 1995. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. *Science (Wash. DC).* 270:797–800.
21. Friedrich, W., C. Knobloch, J. Greher, W. Hartmann, H.H. Peter, S.F. Goldmann, and E. Kleihauer. 1993. Bone marrow transplantation in severe combined immunodeficiency: potential and current limitations. *Immunodeficiency.* 4:315–322.
22. Miller, A.D., and C. Buttimore. 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. *Mol. Cell. Biol.* 6:2895–2902.

23. Miller, A.D., J.V. Garcia, N. von Suhr, C.M. Lynch, C. Wilson, and M.V. Eiden. 1991. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J. Virol.* 65:2220–2224.
24. Markowitz, D., S. Goff, and A. Bank. 1988. Construction and use of a safe and efficient amphotropic packaging cell line. *Virology.* 167:400–406.
25. Sambrook, J., E. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, NY. F1-11.
26. Williams, D.A., K. Hsieh, A. DeSilva, and R.C. Mulligan. 1987. Protection of bone marrow transplant recipients from lethal doses of methotrexate by the generation of methotrexate-resistant bone marrow. *J. Exp. Med.* 166:210–218.
27. Candotti, F., J.A. Johnston, J.M. Puck, K. Sugamura, J.J. O'Shea, and R.M. Blaese. 1996. Retroviral-mediated gene correction for X-linked severe combined immunodeficiency (XSCID). *Blood.* 87:3097–3102.
28. Bunnell, B.A., Muul, L.M., Donahue, R.E., Blaese, R.M., Morgan, R.A. 1995. High-efficiency retroviral-mediated gene transfer into human and non-human primate peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. USA.* 92:7739–7743.
29. Miyazaki, T., A. Kawahara, H. Fuji, Y. Nakagawa, Y. Minami, Z.J. Liu, I. Oishi, O. Silvennoinen, B.A. Witthuhn, J.N. Ihle, and T. Taniguchi. 1994. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. *Science (Wash. DC).* 266:1045–1047.
30. Kawahara, A., Y. Minami, T. Miyazaki, J.N. Ihle, and T. Taniguchi. 1995. Critical role of the interleukin 2 (IL-2) receptor gamma-chain-associated Jak3 in the IL-2-induced c-fos and c-myc, but not bcl-2, gene induction. *Proc. Natl. Acad. Sci. USA.* 92:8724–8728.
31. Buckley, R.H. 1993. Primary Immunodeficiency Diseases. *In* Fundamental Immunology. W.E. Paul, editor. Raven Press, New York, NY. 1353–1374.
32. Fischer, A., P. Landais, W. Friedrich, G. Morgan, B. Gerritsen, A. Fasth, F. Porta, C. Griscelli, S.F. Goldman, R. Levinsky, and J. Vossen. 1990. European experience of bone-marrow transplantation for severe combined immunodeficiency. *Lancet.* 336:850–854.
33. Blaese, R.M., K.W. Culver, A.D. Miller, C.S. Carter, T. Fleisher, M. Clerici, G. Shearer, L. Chang, Y. Chiang, P. Tolstoshev et al. 1995. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science (Wash. DC).* 270:475–480.
34. Williams, D.A., I.R. Lemischka, D.G. Nathan, and R.C. Mulligan. 1984. Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. *Nature (Lond.).* 310:476–480.
35. Kantoff, P.W., A.P. Gillio, J.R. McLachlin, C. Bordignon, M.A. Eglitis, N.A. Kernan, R.C. Moen, D.B. Kohn, S.F. Yu, E. Karson et al. 1987. Expression of human adenosine deaminase in nonhuman primates after retrovirus-mediated gene transfer. *J. Exp. Med.* 166:219–234.
36. Kiem, H.P., B. Darovsky, C. von Kalle, S. Goehle, D. Stewart, T. Graham, R. Hackman, F.R. Appelbaum, H.J. Deeg, A.D. Miller et al. 1994. Retrovirus-mediated gene transduction into canine peripheral blood repopulating cells. *Blood.* 83:1467–1473.
37. Dunbar, C.E., M. Cottler-Fox, J.A. O'Shaughnessy, S. Doren, C. Carter, R. Berenson, S. Brown, R.C. Moen, J. Greenblatt, F.M. Stewart et al. 1995. Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood.* 85:3048–3057.
38. Kohn, D.B., K.I. Weinberg, J.A. Nolta, L.N. Heiss, C. Lenarsky, G.M. Crooks, M.E. Hanley, G. Annett, J.S. Brooks, A. el-Khoureiy et al. 1995. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. *Nat. Med.* 1:1017–1023.
39. Bordignon, C., L.D. Notarangelo, N. Nobili, G. Ferrari, G. Casorati, P. Panina, E. Mazzolari, D. Maggioni, C. Rossi, P. Servida et al. 1995. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. *Science (Wash. DC).* 270:470–475.
40. Thomis, D.C., C.B. Gurniak, E. Tivol, A.H. Sharpe, and L.J. Berg. 1995. Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. *Science (Wash. DC).* 270:794–797.
41. Nosaka, T., J.M. van Deursen, R.A. Tripp, W.E. Thierfelder, B.A. Witthuhn, A.P. McMickle, P.C. Doherty, G.C. Grosveld, and J.N. Ihle. 1995. Defective lymphoid development in mice lacking Jak3. *Science (Wash. DC).* 270:800–802.
42. Park, S.J., K. Saijo, T. Takahashi, M. Osawa, H. Arase, N. Hirayama, K. Miyake, H. Nakauchi, T. Shirasawa, and T. Saito. 1995. Developmental defects of lymphoid cells in JAK3 kinase-deficient mice. *Immunity.* 3:771–782.