Gene Therapy (1999) 6, 1170–1174
http://www.stockton-press.co.uk/gt

BRIEF COMMUNICATION

Retrovirus-mediated WASP gene transfer corrects defective actin polymerization in B cell lines from Wiskott–Aldrich syndrome patients carrying 'null' mutations

F Candotti[1], F Facchetti[2], L Blanzuoli[2], DM Stewart[3], DL Nelson[3] and RM Blaese[1]
[1]*Clinical Gene Therapy Branch, National Human Genome Research Institute,* [3]*Immunophysiology Section, Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD, USA; and* [2]*Department of Pathology, University of Brescia, Spedali Civili, Brescia, Italy*

Boys affected with Wiskott–Aldrich syndrome (WAS) present with variable association of thrombocytopenia, eczema and immune deficiency. If untreated, WAS patients may succumb to intracerebral hemorrhages, severe infections or malignancies. Allogeneic bone marrow transplantation (BMT) can cure all aspects of the disease, but HLA-identical donors are not available to all patients and mismatched BMTs are unfortunately associated with high mortality and morbidity. The good success of HLA-matched BMT, however, makes WAS a potential candidate for hematopoietic stem cell gene therapy. WAS patients carry mutations of the Wiskott–Aldrich syndrome protein gene encoding WASP, a 502-amino acid proline-rich protein with demonstrated involvement in the organization of the actin cytoskeleton. To verify the feasibility of genetic correction for this disease, the WASP cDNA was expressed in EBV-immortalized B cell lines obtained from WAS patients using a retroviral vector. Transduced WAS cells showed levels of WASP expression similar to those found in cells from normal donors, without detectable effects on viability or growth characteristics. In addition, retrovirus-mediated expression of WASP led to improvement of cytoplasmic F-actin expression and formation of F-actin-positive microvilli, a process shown to be defective in untransduced WAS cell lines. These preliminary results indicate a potential use for retrovirus-mediated gene transfer as therapy for WAS.

Keywords: *retroviruses; genetic vectors; gene therapy; cytoskeleton; Wiskott–Aldrich syndrome*

The Wiskott–Aldrich syndrome (WAS) is a severe X-linked disorder characterized by thrombocytopenia with small sized platelets, eczema of the skin, and immunodeficiency leading to increased susceptibility to infection from all classes of pathogens, as well as a high incidence of lymphoid cancer. The clinical spectrum of the disease extends from a full blown presentation of often life-threatening illness, to much milder variants characterized only by isolated thrombocytopenia with small platelets (X-linked thrombocytopenia, XLT). Common immunopathologic findings in 'classical' WAS patients include low serum levels of IgM, and increased IgA and IgE; failure to produce antibodies to polysaccharide antigens, skin test anergy and *in vitro* deficits in T and B cell functions. Sepsis and severe intra-cerebral hemorrhages represent frequent causes of death in WAS boys, and malignancies (mainly non-Hodgkin lymphomas) also occur with increased frequency.[1,2]

Positional cloning has led to the identification of *wasp*, the gene mutated in WAS[3] and XLT patients,[4] which encodes a 502-amino acid (aa) product named the Wiskott–Aldrich syndrome protein (WASP). Studies aimed at understanding the function of WASP have defined a preferential pattern of expression in hematopoietic cells[3,5,6] and identified a number of cellular interacting partners. Several SH3 domain-containing proteins, including adaptors and cellular kinases (Nck, Fyn, Tec, Itk, Btk, PLC-γ, C-Fgr, Grb-2), have been shown to bind WASP *in vitro* and *in vivo* (reviewed in Ref. 2), thus indicating a potential role for WASP in cytokine and growth factor signal transduction pathways. Evidence of a link between WASP and cytoskeleton re-organization has also derived from studies demonstrating the interaction between WASP and the Cdc42 and Rac small GTPases.[7–9] Further evidence includes the induction of actin-containing clusters in cells transfected or micro-injected with WASP[8] or WIP, a recently identified WASP-interacting protein.[10] Although the definitive function of WASP remains to be determined, a tentative unifying interpretation of the currently available knowledge would suggest that WASP plays a critical role in cytoskeleton organization of hematopoietic cells (megakaryocytes, lymphocytes), possibly in response to cellular activation.

All clinical aspects of the disease are curable by allo-

Correspondence: F Candotti, Clinical Gene Therapy Branch, National Human Genome Research Institute, National Institutes of Health, 10 Center Drive, Building 10, Room 10C103, Bethesda, MD 20892-1851, USA
Received 15 October 1998; accepted 5 February 1999

geneic bone marrow transplantation (BMT) with a success rate of approximately 90% when histocompatible sibling donors are available,[11] or when matched unrelated (MUD) transplants are performed in the first years of life. Conversely, MUD BMTs in older WAS patients and T cell-depleted haploidentical transplants have much less satisfactory outcomes with long-term survival ranging from approximately 15% to approximately 45% complicated by high incidence of graft-versus-host reactions.[12] Unfortunately, HLA-identical donors are not available to the majority of those severely affected WAS patients who are candidates for BMT. These patients could benefit from the development of gene-based therapeutic strategies and the recent identification of the molecular basis of this disorder has opened the way to preliminary testing.

A retroviral vector (GCWASP) which carries the human WASP cDNA (generously provided by Dr S Weissman, Yale University, New Haven, CT, USA) and the neomycin resistance gene (*neo*) was constructed (Figure 1) and packaged in the gibbon-ape leukemia virus (GALV) envelope using the PG13 cell line.[13] A second retroviral construct (pSAM-EN)[14] sharing the same backbone, but exclusively expressing *neo* was used as negative control. Epstein–Barr virus (EBV)-immortalized B cell lines (BCLs) were grown from WAS patients with null mutations of WASP and transduced with retroviral supernatants following an optimized gene transfer protocol.[15] After transduction, cells were subjected to G418 selection and analyzed for WASP expression by Western blot technique using a WASP-specific monoclonal antibody.[5] As shown in Figure 2a, BCLs from a normal control individual (lane 1) showed the presence of an approximately 59 kDa WASP band which was not detectable in untreated BCLs from two WAS patients, or WAS-BCLs transduced with the SAM-EN control vector (lanes 2, 3 and 5, 6). Conversely, levels of WASP expression comparable with normal were demonstrated in cell lysates from GCWASP-transduced WAS BCLs (lanes 4 and 7). Similar results were obtained after transduction and Western blot analysis of an additional four BCLs obtained from unrelated WAS patients (data not shown). To verify further the physical characteristics of

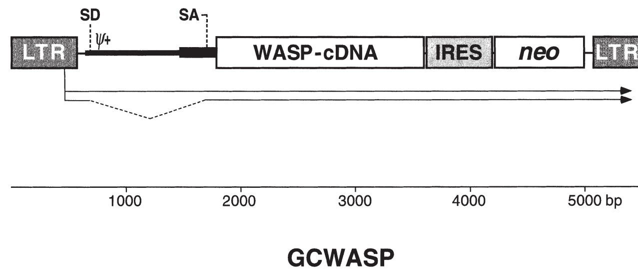

Figure 1 *Schematic representation of the GCWASP retroviral vector. A copy of the complete WASP open reading frame was subcloned into the SAM-EN retroviral cassette[14] using the NotI and SnaBI sites. Infectious retroviral particles packaged into the gibbon-ape leukemia virus (GALV) envelope were produced using the PG13 packaging cell line[13] following procedures previously described.[26] GCWASP and SAM-EN retroviral supernatants were obtained from cells cultured in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 2* mm *l-glutamine (both from Life Technologies) and 50 μg/ml gentamycin (Bio-Whittaker, Walkerswille, MD, USA). These vector supernatants were then used to transduce EBV-immortalized lymphoblastoid cells obtained from WAS patients, following an 'optimized' transduction protocol involving phosphate depletion, centrifugation, and low temperature (32°C) incubation.[15] Briefly, after incubation in phosphate-free RPMI-1640 (Life Technologies) for 6–9 h, 5×10^6 cells were resuspended in retroviral supernatant supplemented with 8 μg/ml polybrene (Sigma, St Louis, MO, USA), transferred to 24-well plates and spun at 1800 g for 30 min, at 32°C. Cells were then incubated at 37°C overnight. The following day, the same procedure was repeated. This transduction protocol allows us to routinely achieve transduction efficiencies of 5–10% in EBV-B cell lines, as estimated by DNA-PCR 48 h after vector exposure (data not shown). After transduction, cells were selected in 1.0 mg/ml of neomycin analog, G418 (Geneticin; Life Technologies) for 10–14 days. Lymphoblastoid cells were maintained in RPMI 1640, 10% FBS, 2* mm *l-glutamine (all from Life Technologies) and 50 μg/ml gentamycin (Bio-Whittaker). LTR, long terminal repeat; ψ+, packaging signal sequence; SD, splice donor site; SA, splice acceptor site; IRES, encephalomyocarditis virus internal ribosomal entry site sequence; neo, neomycin resistance gene.*

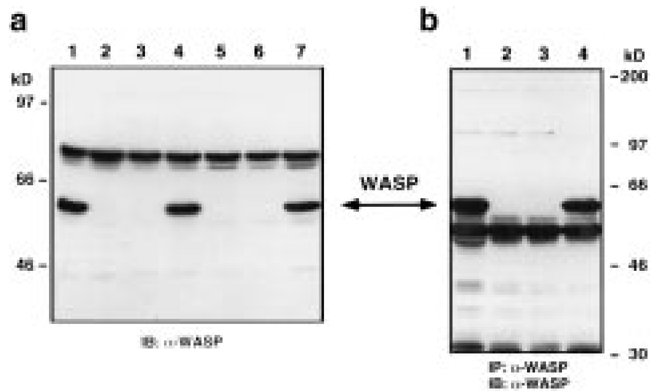

Figure 2 *(a) Western blot analysis of retrovirus-mediated expression of WASP. Cells were lysed in buffer containing 300* mm *NaCl, 50* mm *Tris-HCl, 2* mm *EDTA, 0.5% Triton X-100, 2.5 μm p-nitrophenyl p′-guanidino-benzoate, 10 μg/ml aprotinin, and leupeptin, and protein concentration determined using the BCA protein assay (Pierce, Rockford, IL, USA). One hundred micrograms of protein were then boiled, subjected to 8% SDS-PAGE, and electro-transferred on to nylon membranes (Immobilon-P; Millipore, Bradford, MA, USA) which were first incubated for 1 h in 'blocking buffer' (20* mm *Tris, 150* mm *NaCl, 1% fish gelatine, 2% goat serum, 0.1% BSA, 0.5% Tween 20) and then immunoblotted (IB) with a mouse monoclonal Ab specific for the human WASP[5] (α-WASP) diluted 1:10 000 in 'blotting buffer' (20* mm *Tris, 150* mm *NaCl, 1% fish gelatine, 0.1% BSA, 0.1% Tween 20) also for 1 h. After washing in TBS-T buffer (20* mm *Tris, 150* mm *NaCl, 0.1% Tween 20) a biotinylated goat anti-mouse secondary antibody (Oncogene, Cambridge, MA, USA) was then diluted (1:8000) in 'blocking buffer' and applied for 1 h. Following further washing in TBS-T, the membrane was incubated in a 1:20 000 dilution of horseradish peroxidase-conjugated streptavidin for 30 min and then extensively washed in TBS-T. Detection of immune complexes was performed by enhanced chemiluminescence (ECL; Amersham Life Science, Arlington Heights, IL, USA). The membrane was then stripped and re-probed with a mouse monoclonal antibody specific to β-actin (Sigma), to verify equal loading (data not shown). Lane 1, lysate of BCL from normal control; lanes 2 and 5, BCLs from WAS patients 1 and 2, respectively; lanes 3 and 6, BCLs from WAS patients 1 and 2 transduced with the SAM-EN control vector; lanes 4 and 7, BCLs from WAS patients 1 and 2 transduced with GCWASP. (b) Immunoprecipitation (IP) of WASP from normal control and GCWASP retrovirally transduced WAS BCLs. Cells (1×10^7) were lysed in the above defined lysis buffer and, after centrifugation, postnuclear supernatants were immunoprecipitated with α-WASP bound to protein G-sepharose (Pharmacia Biotech, Piscataway, NJ, USA). Immunoprecipitates were then washed, boiled, and resolved using 12% SDS-PAGE electrophoresis. After electrotransfer, membranes were immunoblotted with α-WASP and detection was performed by ECL. Lane 1, BCL from normal control; lane 2, BCL from WAS patient 2; lane 3, BCL from WAS patient 2 transduced with the SAM-EN control retroviral vector; lane 4, BCL from WAS patient 2 transduced with the GCWASP retroviral vector. Standard molecular weights are indicated (kD).*

the transduced WASP protein, we performed immunoprecipitation experiments on WAS BCLs before and after corrective gene transfer. Figure 2b shows the results of a representative experiment demonstrating that the retrovirally expressed WASP was appropriately recognized in liquid phase by specific monoclonal antibodies (lane 4), closely reproducing the finding in BCL from normal donors (lane 1). As expected, no product was precipitable from cell lysates of untransduced WAS BCL (lane 2) nor from the same cells transduced with the control SAM-EN vector (lane 3).

Cell surface defects in WAS blood lymphocytes suggesting impairment of cytoskeletal organization have been described since the mid 1980s, well before the gene responsible for the disease was identified.[16] More recently, the demonstration of the interaction of WASP with key regulators of cytosketal reorganization, Cdc42 and rac,[7–9] has led to further analysis of the cytoskeleton in WAS cells. These studies have identified abnormal actin rearrangement in WAS T lymphocytes in response to CD3-mediated stimulation,[17] as well as defective filamentous actin (F-actin) polymerization and alpha-actinin distribution in WAS lymphoblastoid cell lines.[18]

Because of the limited current knowledge of the specific function of WASP, no assays are available to test directly the function of the retrovirally transduced protein. We therefore chose to observe the effects of WAS gene expression on some of the cytoskeletal abnormalities described in WAS BCLs. Samples were processed and scored (Table 1) by blinded operators. As described in Figure 3, studies of F-actin distribution in BCLs from normal donors stained with FITC-conjugated phalloidin (FP) showed diffused cytoplasmic reactivity in addition to strong linear positivity at the level of microvilli (Figure 3a). In keeping with previous observations,[18] cytoplasmic distribution of F-actin was markedly reduced or abolished in WAS BCLs. In addition, WAS BCLs showed limited numbers of F-actin-positive microvilli which appeared structurally abnormal compared with normal

Table 1 Analysis of F-actin distribution by phalloidin-FITC staining

Sample	*Cytoplasmic positivity (%)*	*Cells with F-actin-positive complete microvilli (No.)*
Normal controls	40–90	tnc
WAS1	10	None
WAS1/SAM-EN	5–10	None
WAS1/GCWASP	50–60	5
WAS2	<10	None
WAS2/GCWASP	40–50	3

Cytospin preparations, staining, and analysis were performed by blinded operators as described in the legend to Figure 3. Cytoplasmic reactivity to phalloidin-FITC was expressed as the percentage of total cells present in the cytospin evaluated at a ×40 magnification. The number of cells carrying F-actin-positive microvilli was counted in 10 fields using a ×100 microscope objective. BCL from WAS patient 1 were analyzed before (WAS1) and after gene transduction with the SAM-EN control retroviral vector (WAS1/SAM-EN) or the GCWASP construct (WAS1/GCWASP). BCL from WAS patient 2 were studied before (WAS2) and after gene transfer with GCWASP vector (WAS2/GCWASP).
tnc, too numerous to count.

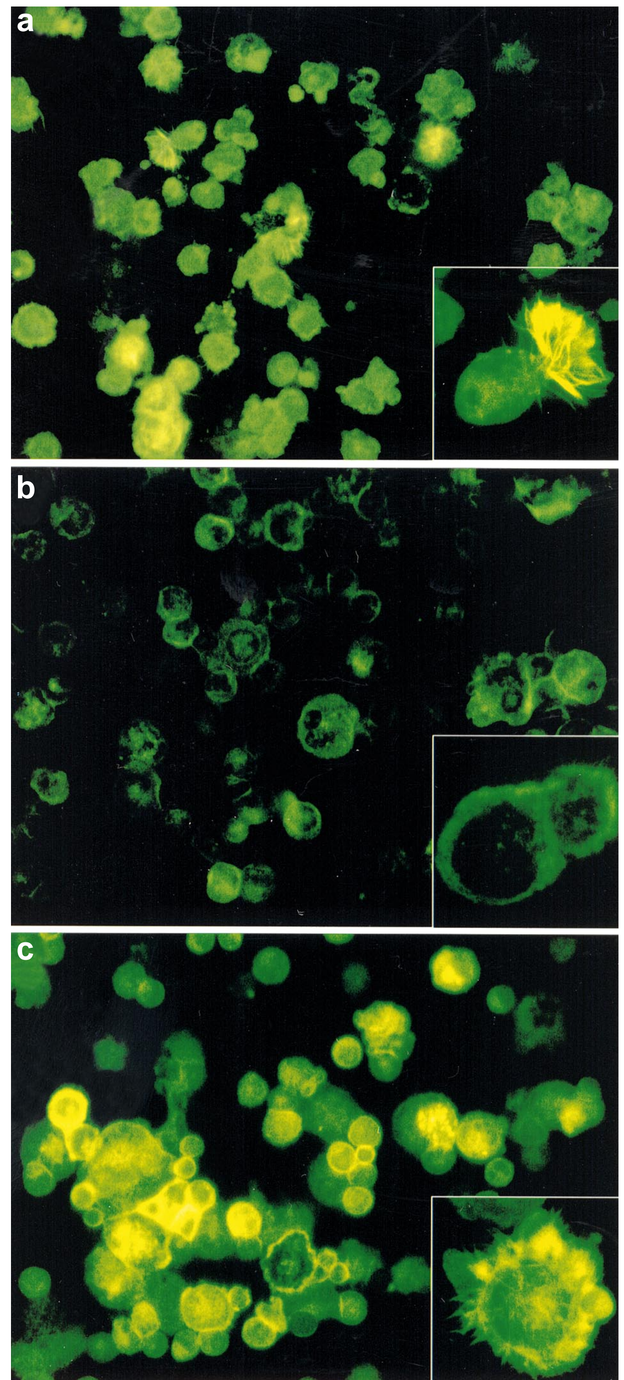

Figure 3 *Representative images of assessment of F-actin distribution before and after WASP gene transfer. Visualization of F-actin was performed as described.[18] Briefly, cells were washed and adjusted to a concentration of 5×10^6/ml. One hundred microliters were then used to prepare cytospin slides which were air-dried and fixed in absolute ethanol at –20°C. F-actin was visualized using FITC-conjugated phalloidin (Sigma) which was applied at a 1:5 dilution for 30 min. Slides were then analyzed with a Jenamed-2 fluorescence microscope. (a) Normal control BCL showing cytoplasmic and microvillar (inset) expression of F-actin in most cells. (b) WAS patient 2 BCL demonstrating clear reduction of F-actin cytoplasmic distribution. Incomplete, blunted microvilli characterized by only weak F-actin reactivity are observed at higher magnification (inset). (c) WAS patient 2 BCL after GCWASP transduction showing marked increase of F-actin reactivity and presence of cells carrying F-actin-positive microvilli (inset). Similar results were obtained from the analysis of BCL from WAS patient 1 (data not shown). Panel a, b and c: × 40; insets: × 100.*

controls (Figure 3b). In marked contrast, FP staining of WASP-transduced WAS BCLs demonstrated a clear increase of cytoplasmic F-actin to levels comparable with BCLs from normal controls. In addition, we observed the presence of some mature actin-filled microvilli which were not detectable before gene transfer (Figure 3c). WAS BCLs transduced with the SAM-EN control vector did not show improvement of F-actin distribution and were comparable with untransduced cells (data not shown).

Our results demonstrate that retrovirus-mediated gene transfer can reconstitute normal levels of expression of WASP protein in lymphocytic lines obtained from patients affected with WAS. In addition we show that '*de novo*' expression of the WASP protein leads to restoration of normal F-actin cytoplasmic distribution in WAS BCLs. Alhough indirect, this evidence argues for adequate functionality of the newly expressed protein and indicates that gene addition strategies may be exploited for correction of biological defects characteristic of WAS cells. Only a few of the transduced WAS BCLs showed the appearance of mature F-actin-containing microvilli. The reasons underlying these findings are presently unclear. It is possible that the ability of WAS EBV-B cells to produce specialized structures such as microvilli cannot be restored by ectopic expression of WASP or that WASP was not expressed at sufficient levels. Further studies are underway in an attempt to address these issues.

Unregulated expression of WASP has been associated with toxic effects of target cells[8] thus generating concern as to potential detrimental effects of WASP 'ectopic' expression following retroviral transduction. We have generated several NIH 3T3-based retroviral producer clones as well as many WAS BCLs expressing WASP under the transcriptional control of the Moloney murine leukemia virus (MoLV) and have not observed any negative metabolic effects on the transfected/transduced cells. The lack of toxicity in our studies may reflect different levels of WASP expression related to lower *wasp* copy number per cell or to reduced transcription mediated by the MoLV LTR. Of note, the level of expression of WASP in our transduced WAS BCLs was similar to that physiologically expressed by BCLs from normal controls (Figure 2), whereas the levels of WASP associated with cellular toxicity were achieved only after WASP overexpression.[8]

Our current limited knowledge of the regulation of the *wasp* gene and the specific function(s) of the WASP protein poses obvious limitations to the development of clinical applications of gene therapy for this disease, and a better understanding of the mechanisms linking mutations in the *wasp* gene to the WAS phenotype(s) is needed before corrective gene transfer can be proposed as a treatment for WAS patients. Murine 'knock-out' models of WAS have recently been generated which show some of the biological defects observed in humans[19] and these animals will constitute an important tool for the assessment of safety and efficacy of gene-based therapies. Moreover, studies need to be performed on patient cells with residual expression of mutated WASP to determine whether 'gene addition' strategies may be hindered by dominant negative effects of the mutant protein over the transduced normal WASP.

Because WAS affects multiple hematopoietic cell types and since allogeneic BMT cures all aspects of the diseases, the hematopoietic stem cell is the ultimate target for corrective gene transfer. Retrovirus-mediated transfer of the WASP cDNA into hematopoietic progenitors of WAS patients does not seem to induce negative effects on differentiation of stem cells, as recently reported in abstract form by Huang *et al*.[20] The experience from allogeneic BMT for WAS indicates that myeloablation is a necessary prerequisite to successful engraftment of normally WASP-expressing hematopoietic stem cells.[12,21] This observation suggests that in a gene therapy setting, gene-corrected hematopoietic progenitors would not have selective advantage over unmodified stem cells and that myeloblation would probably still be required before re-infusion of genetically engineered cells in order to achieve clinical benefit. In this scenario, high efficiency gene transfer into the hematopoietic stem cells is critical and probably beyond our current technical abilities. '*In vivo*' positive selection of transduced progenitors could represent a way to overcome this problem and could be achieved by adding *in vivo* selectable drug resistance genes (eg MDR, DHFR) to the retroviral vector cassette.

From the analysis of the natural history of the disease, it appears clear that, once the threat of lethal cerebral bleeding threatening mostly infants and young children is reduced, the main causes of death in WAS patients are represented by autoimmunity, sepsis and cancer. Studies of T lymphocytes and T cell lines obtained from WAS patients have demonstrated *in vitro* defects of antigen receptor-mediated activation and actin polymerization,[17,22] while transmembrane signaling seems to be defective in lymphoblastoid WAS B cell lines,[23] but not in freshly isolated B lymphocytes.[24] In addition, WASP 'knock-out' mice show defective antigen receptor-induced proliferation in T, but not B lymphocytes.[20] Defective T cell immune surveillance against infections and malignancies may account for the clinical complications appearing in WAS patients in late childhood, adolescency and adulthood, and, therefore, T lymphocyte-directed gene therapy could be proposed as an attempt to restore T cell functions in patients for whom BMT is not available. In such an approach, the inclusion in the vector design of 'fail-safe' mechanisms, such as herpes simplex thymidine kinase,[25] might be used to allow elimination of transduced cells in the case of unwanted consequences of WASP expression. Our experiments are particularly relevant to this respect since they demonstrate the feasibility of '*in vitro*' corrective gene transfer into mature patients' lymphoid cells and form the basis for further evaluations of therapeutic approaches for WAS based on retrovirus-mediated gene transfer.

Acknowledgements

We are grateful to Dr S Weissman for donating the WASP cDNA and to Prof LD Notarangelo for insightful discussion and continuous support.

References

1 Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott–Aldrich syndrome. *J Pediatr* 1994; **125**: 876–885.
2 Ochs HD. The Wiskott–Aldrich syndrome. *Springer Semin Immunopathol* 1998; **19**: 435–458.
3 Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. *Cell* 1994; **78**: 635–644.

4 Villa A *et al*. X-linked thrombocytopenia and Wiskott–Aldrich syndrome are allelic diseases with mutations in the WASP gene. *Nat Genet* 1995; **9**: 414–417.
5 Stewart DM *et al*. Studies of the expression of the Wiskott–Aldrich syndrome protein. *J Clin Invest* 1996; **97**: 2627–2634.
6 Parolini O *et al*. Expression of Wiskott–Aldrich syndrome protein (WASP) gene during hematopoietic differentiation. *Blood* 1997; **90**: 70–75.
7 Aspenstrom P, Lindberg U, Hall A. Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome. *Curr Biol* 1996; **6**: 70–75.
8 Symons M *et al*. Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell* 1996; **84**: 723–734.
9 Kolluri R *et al*. Direct interaction of the Wiskott Aldrich syndrome protein with the GTPase Cdc42. *Proc Natl Acad Sci USA* 1996; **93**: 5616–5618.
10 Ramesh N, Anton IM, Hartwig JH, Geha RS. WIP, a protein associated with Wiskott–Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. *Proc Natl Acad Sci USA* 1997; **94**: 14671–14676.
11 Brochstein JA *et al*. Marrow transplantation from human leukocyte antigen-identical or haploidentical donors for correction of Wiskott–Aldrich syndrome. *J Pediatr* 1991; **119**: 907–912.
12 Filipovich A. Stem cell transplantation from unrelated donors for correction of primary immunodeficiencies. *Immunol All Clin N Am* 1996; **16**: 377–392.
13 Miller AD *et al*. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J Virol* 1991; **65**: 2220–2224.
14 Treisman J *et al*. Interleukin-2-transduced lymphocytes grow in an autocrine fashion and remain responsive to antigen. *Blood* 1995; **85**: 139–145.
15 Bunnell BA *et al*. High-efficiency retrovirus-mediated gene transfer into human and nonhuman primate peripheral blood lymphocytes. *Proc Natl Acad Sci USA* 1995; **92**: 7739–7743.
16 Kenney D *et al*. Morphological abnormalities in the lymphocytes of patients with the Wiskott–Aldrich syndrome. *Blood* 1986; **68**: 1329–1332.
17 Gallego MD, Santamaria M, Pena J, Molina IJ. Defective actin reorganization and polymerization of Wiskott–Aldrich T cells in response to CD3-mediated stimulation. *Blood* 1997; **90**: 3089–3097.
18 Facchetti F *et al*. Defective actin polymerization in EBV-transformed B-cell lines from patients with the Wiskott–Aldrich syndrome. *J Pathol* 1998; **185**: 99–107.
19 Snapper SB *et al*. Wiskott–Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* 1998; **9**: 81–91.
20 Huang MM *et al*. Expression of human Wiskott–Aldrich-syndrome protein in patients primary hematopoietic-cells and Ebv-transduced cell-lines after retroviral vector-mediated gene transduction. *Blood* 1997; **90**: 1154.
21 Fischer A *et al*. Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. *Blood* 1994; **83**: 1149–1154.
22 Molina IJ *et al*. T cells of patients with the Wiskott–Aldrich syndrome have a restricted defect in proliferative responses. *J Immunol* 1993; **151**: 4383–4390.
23 Simon HU, Mills GB, Hashimoto S, Siminovitch KA. Evidence for defective transmembrane signaling in B cells from patients with Wiskott–Aldrich syndrome. *J Clin Invest* 1992; **90**: 1396–1405.
24 Henriquez NV, Rijkers GT, Zegers BJ. Antigen receptor-mediated transmembrane signaling in Wiskott–Aldrich syndrome. *J Immunol* 1994; **153**: 395–399.
25 Tiberghien P *et al*. Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: an approach for specific *in vivo* donor T-cell depletion after bone marrow transplantation? *Blood* 1994; **84**: 1333–1341.
26 Candotti F *et al*. Retroviral-mediated gene correction for X-linked severe combined immunodeficiency. *Blood* 1996; **87**: 3097–3102.