Check for updates

(allogeneic transplant), 40% (autologous transplant), and 61% (intensive chemotherapy).[2] These numbers are significantly different from the results published in 1996, and compare quite favorably with those reported by Kawasaki et al (72%-74% 3-year survival for a total of 35 patients). While the use of the more recently published CCG results does not change substantively the results or conclusions reached by Kawasaki et al, comparison with the more recent results provides a more appropriate context for evaluating the significance of their findings. These studies complement each other, providing evidence that an age of younger than 1 year is probably not an independent adverse prognostic factor for children with AML, supporting the use of intensive chemotherapy when there is no allogeneic bone marrow donor available.

David M. Loeb and Robert J. Arceci

Correspondence: Robert J. Arceci, Division of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD 21231

References

1. Kawasaki H, Isoyama K, Eguchi M, et al. Superior outcome of infant acute myeloid leukemia with intensive chemotherapy: results of the Japan Infant Leukemia Study Group. Blood. 2001;98:3589-3594.
2. Woods WG, Neudorf S, Gold S, et al. A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission: a report from the Children's Cancer Group. Blood. 2001;97:56-62.

To the editor:

Selective serotonin reuptake inhibitors are effective in the treatment of polycythemia vera–associated pruritus

Polycythemia vera is a clonal hematopoietic stem cell disorder characterized by erythropoietin-independent erythrocytosis that is often accompanied by splenomegaly, thrombocytosis, and leukocytosis.[1] The disease is associated with life-threatening thrombohemorrhagic complications and a progressive risk of transformation to either myelofibrosis with myeloid metaplasia or acute myeloid leukemia. Pruritus, which is often exacerbated by contact with water, occurs in more than 50% of patients with polycythemia vera and may be the most agonizing aspect of the disease, depriving patients of sleep and interfering with their social and physical activities.[2] Phlebotomy with or without cytoreductive therapy, the standard treatment for polycythemia vera, is often ineffective in alleviating disease-associated pruritus. More recently, the use of interferon alpha as a cytoreductive agent in polycythemia vera has proven particularly effective in controlling pruritus.[3] However, cytoreductive treatment in polycythemia vera is generally reserved for high-risk patients, and the antipruritic benefit of interferon alpha is undermined by the well-known side effects of the drug. On the other hand, symptomatic treatment with antihistamines is often ineffective.

On the basis of a serendipitous anecdotal observation as well as reports of efficacy in pruritus associated with advanced cancer,[4] 10 patients (median age, 68 years; 3 female) with polycythemia vera–associated intractable pruritus were treated with selective serotonin reuptake inhibitors. Nine patients received paroxetine (20 mg/d) and 1 received fluoxetine (10 mg/d). All 10 patients had a favorable initial response, which included complete or near-complete resolution of pruritus in 8 patients (80%). Response to treatment occurred within 48 hours in most patients. Two patients have discontinued treatment because of either side effects or leukemic transformation. One of these 2 patients had a partial relapse (loss of "best" response but severity of pruritus still better than baseline) of her pruritus before discontinuing treatment with paroxetine. Eight patients are still on treatment for a period of 1 to 12 months. Among these patients, 3 have experienced side effects including delayed ejaculation, decreased libido, and fatigue. Only 1 patient has had a partial relapse in pruritus.

The current report suggests that a selective serotonin reuptake inhibitor may be considered as a therapeutic option in polycythemia vera–associated pruritus. A prospective, controlled treatment trial that is accompanied by laboratory correlative studies is required to validate the current preliminary observations as well as clarify the mechanism of action.

Ayalew Tefferi and Rafael Fonseca

Correspondence: Ayalew Tefferi, Division of Hematology and Internal Medicine, Mayo Clinic, Rochester, MN

Both authors contributed equally to the work.

References

1. Tefferi A, Solberg LA, Silverstein MN. A clinical update in polycythemia vera and essential thrombocythemia. Am J Med. 2000;109:141-149.
2. Diehn F, Tefferi A. Pruritus in polycythaemia vera: prevalence, laboratory correlates and management. Br J Haematol. 2001;115:619-621.
3. Finelli C, Gugliotta L, Gamberi B, Vianelli N, Visani G, Tura S. Relief of intractable pruritus in polycythemia vera with recombinant interferon alfa. Am J Hematol. 1993;43:316-318.
4. Zylicz Z, Smits C, Krajnik M. Paroxetine for pruritus in advanced cancer. J Pain Symptom Manage. 1998;16:121-124.

To the editor:

The Fanconi anemia cell line HSC536N is not sensitive to interferon-γ and does not cleave PARP in response to Fas-mediated cell killing

Rathbun et al[1] have reported apoptosis in the Fanconi anemia (FA) cell line HSC536N in response to interferon-γ (IFN-γ) and agonistic Fas antibodies. While they did not use the classic assays of apoptosis (morphology, TUNEL, DNA laddering), they concluded that cell death was due to apoptosis because of evidence of both caspase 3 and PARP cleavage on Western blots. In order to show cleavage, however, they had to load 100 μg of protein per lane onto the gel and, allowing for overloading, the

Downloaded from http://ashpublications.org/blood/article-pdf/99/7/2627/1682299/2627a.pdf by guest on 25 June 2024

Table 1. Levels of cell death and apoptosis measured by 7AAD staining in HSC536N cells treated for 6 hours with IFN-γ with or without anti-Fas antibodies (mean of 3 experiments)

	Levels of cell death and apoptosis								
Time (h)	0	6	6	6	6	6	6	6	6
IFN-γ (ng/mL)	0	0	0.1	1	10	0	0.1	1	10
Anti-Fas (ng/mL)	0	0	0	0	0	250	250	250	250
% 7AAD positive (mean ± SEM)	18 ± 3.4	16 ± 1.9	16 ± 2.3	16 ± 0.8	15 ± 2.0	35 ± 1.6	33 ± 2.5	31 ± 3.5	31 ± 2.7

blots appear to show substantially less than 1% cleavage. In our experience, substantial cleavage of caspase 3 during apoptosis can be seen at standard loadings of 10 μg or less, and PARP cleavage is often quantitative in nature. Since we and others have shown that FA cell lines do not undergo normal apoptosis in response to mitomycin C,[2,3] we were interested whether the reported conclusions were correct.

We incubated the *FANCC* mutant cell line HSC536N for 6 hours with different doses of IFN-γ, with or without an agonistic Fas antibody, and measured cell death by 7-amino actinomycin D (7AAD) staining and flow cytometry, which detects early changes in membrane permeability.[4] Surprisingly, we found no response to IFN-γ alone and no enhancement of Fas-mediated killing (Table 1).

Cell extracts were subjected to Western blotting and probed with an antibody for caspase 3 (Pharmingen, catalog no. 65906E, San Diego, CA) (Figure 1). In contrast to Rathbun et al, we found substantial cleavage of caspase 3, with generation of p20 and p17 cleaved forms, with only 10 μg protein loading. To further investigate the discrepancy with the previous report, we repeated the blots using the same antibody employed by Rathbun et al (Transduction Laboratories, C31720, San Diego, CA). Cleaved forms of caspase 3 were scarcely detectable at 10 μg loading (Figure 1B). We note that the manufacturer only claims detection of the proform of caspase 3 by this antibody. Western blots were also performed with an anti-PARP antibody. In this case we found no detectable evidence of PARP cleavage, compared to standard controls (Figure 1). DNA was also extracted for gel electrophoresis, and DNA laddering was not detected.

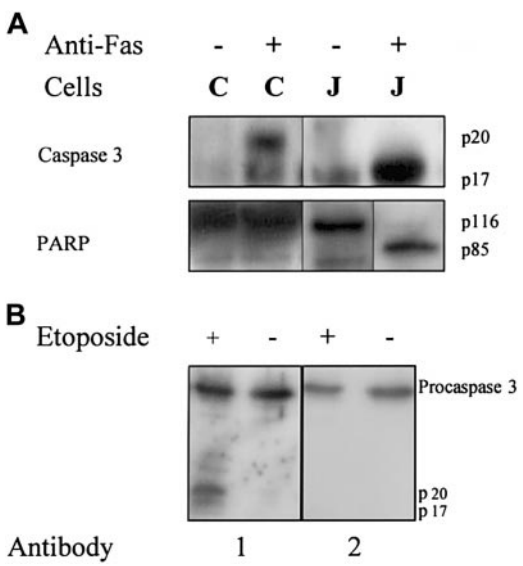

Figure 1. Western blotting to detect cleaved forms of caspase 3 and PARP. (A) HSC536N cells (C) and Jurkat cells (J) were treated with anti-Fas antibody for 6 hours and analyzed for the p20 and p17 cleaved forms of caspase 3 (top), and the p85 cleaved form of PARP (bottom). Note an artefact band migrating faster than p85 PARP is present in treated and untreated cells. (B) Jurkat cells were treated with etoposide for 6 hours and analyzed for the p20 and p17 cleaved forms of caspase 3 using Pharmingen antibody catalog no. 65906E[1] or Transduction Laboratories antibody C31720.[2]

Are these cells really undergoing apoptosis? The primary definition of apoptosis is based on morphology. Examination of Giemsa-stained cytocentrifuge preparations of anti-Fas–treated cells clearly showed classical apoptosis, with nuclear condensation, and chromatin marginalization and fragmentation. We conclude that HSC536 cells do indeed undergo morphological apoptosis in response to Fas antibodies and that the failure of Rathbun et al to detect quantitative caspase 3 cleavage was a technical problem associated with the antibody. On the other hand, even if the minor band seen in their blots is a PARP cleavage product, cleavage of PARP within these cells is still negligible.

There is clear evidence that primary FA cells are hypersensitive to IFN-γ and that HSC536 cells show biochemical abnormalities in their response to IFN-γ. However, we could not detect any enhancement of Fas-mediated cell death by IFN-γ.[5] We suggest this is because of the high constitutive level of Fas expression on these cells.

Tim R. Rutherford, Nyree E. Myatt, Frances M. Gibson, and Alan A. Clarke

Correspondence: Tim R. Rutherford, Dept of Haematology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE United Kingdom; e-mail: trutherf@sghms.ac.uk

Supported by a grant from the United Kingdom Leukaemia Research Fund

References

1. Rathbun RK, Christianson TA, Faulkner GR, et al. Interferon-gamma-induced apoptotic responses of Fanconi anemia group C hematopoietic progenitor cells involve caspase 8-dependent activation of caspase 3 family members. Blood. 2000;96:4204-4211.
2. Clarke AA, Philpott NJ, Gordon-Smith EC, Rutherford TR. The sensitivity of Fanconi anaemia group C cells to apoptosis induced by mitomycin C is due to oxygen radical generation, not DNA crosslinking. Br J Haematol. 1997;96:240-247.
3. Guillouf C, Wang TS, Liu J, et al. Fanconi anemia C protein acts at a switch between apoptosis and necrosis in mitomycin C-induced cell death. Exp Cell Res. 1999;246:384-394.
4. Philpott NJ, Turner AJ, Scopes J, et al. The use of 7-amino actinomycin D in identifying apoptosis: simplicity of use and broad spectrum of application compared with other techniques. Blood. 1996;87:2244-2251.
5. Pang Q, Fagerlie S, Christianson TA, et al. The Fanconi anemia protein FANCC binds to and facilitates the activation of STAT1 by gamma interferon and hematopoietic growth factors. Mol Cell Biol. 2000;20:4724-4735.

Downloaded from http://ashpublications.org/blood/article-pdf/99/7/2627/1682299/2627a.pdf by guest on 25 June 2024

Response:

Apoptosis and FANCC

Rutherford et al have stated that in our paper on caspase activation in Fanconi anemia (FA)[1] cells "the classic assays of apoptosis" were not used. They are incorrect. Figure 3 was derived from experiments using the TUNEL assay. An article by our group that was published in *Blood* a year earlier[2] also reported our experience using both morphology and TUNEL assays. In both papers we demonstrated that FA cells are excessively apoptotic. Since the publication of the paper in question we have published additional papers that have further clarified the molecular nature of the apoptotic FA-C phenotype[3-8] and have identified a pivotal control point for caspase 3 activation in FA cells.[6,7]

IFN itself cannot fully reveal a differentially hypersensitive FA phenotype in the isogenic cell lines. We reported this a number of years ago when we noted that a blocking antibody to fas abrogated apoptotic responses of FA-C cells to IFNγ indicating that fasL in cocultured cells contributed to what appeared to be simply an IFN effect.[2] Later we reported that the combinations IFNγ and fasL, IFNγ and TNFα, or IFNγ and dsRNA all reveal exaggerated responses in FA-C cells[6-8]; that the double-stranded RNA-dependent protein kinase, PKR, is constitutively activated and excessively induced by cytokines in FA cells[6]; and that the role played by FANCC in modulating PKR activity depends upon its capacity to bind to hsp70, a known modulator of PKR activity.[7] In fact, PKR is a bottleneck point for the apoptotic pathways because a dominant negative inhibitor of PKR abrogates all differential cytokine responses between FA cells and normal cells.[6] Because IFNγ induces expression of PKR, and TNF/fas/dsRNA activate the molecule, IFN functions as an enhancer of PKR activity.

In our hands, the most reliable and quantitative assessments of the induced apoptotic phenotype in FA cells (murine or human cells) are made using a flow cytometric method that permits quantification of single cells containing caspase 3.[6-8] Using this method, TNFα, fas-ligand, and double-stranded RNA induce more apoptotic responses in FA cells pretreated with IFN than in normal cells treated with IFN.[1,6-8] In view of the consideration by Rutherford et al that "classic assays" for apoptosis include TUNEL and morphological analyses, we are not sure why they chose to use 7-AAD staining as their litmus test.

Rutherford et al have stated that "in order to show cleavage," our group "had to load 100 μg of protein." This, too, is incorrect. At no time have we stated that detection of cleavage of either PARP or caspase 3 required a 100 μg load. Also, the authors' statements that "in contrast to Rathbun et al, we found substantial cleavage of caspase 3. . .with only 10 μg loading," and "failure of Rathbun et al to detect quantitative caspase 3 cleavage. . ." are misleading. Their observation does not stand in contrast to ours. We consistently find differential caspase 3 activation and over the past several years have shown that caspase 3 activation can be shown at a single-cell level, a quantitative standard that simply cannot be matched by Western blotting. Indeed, the result reported here by Rutherford et al at once confirms our results and clarifies that the anti-PARP antibody in question is capable of detecting the cleaved form of the molecule. While we have not seen differential PARP activation in isogenic cell lines, we view the immunoblot method as insensitive and have not focused much attention on PARP recently because we now recognize that PARP cleavage is not necessary for execution of an apoptotic program.[9]

The authors' claim that FA cell lines treated with MMC do not undergo "normal apoptosis" is difficult to understand in part because the only cited reference that used isogenic FA-C cells did, in fact, demonstrate clearly that PARP cleavage induced by MMC was noted only in mutant cells, not in FANCC corrected cells.[10(Fig5C)] Moreover, work from our laboratory published in this journal shows a major reduction in TUNEL-positive MMC-exposed cells in FANCC-complemented FA-C cells.[2(Fig6A)] Of equal relevance we have also noted (G.C.B. and R. K. Rathbun, unpublished observations, September 2000) that MMC-exposed FA cells have more cleaved caspase 9 than do MMC-exposed FA-C cells complemented with FANCC.

Because there is not much disagreement on the involvement of caspase 3 in the FANCC pathway, it is probable that all of us share the view that control points for caspase 3 activation are critically important for an understanding of FA cells. We have chosen this path.[1,3,5-8] What are we left with? We are possibly left with some evidence that internucleosomal cleavage of chromatin is somehow different in FA cells treated with MMC, but we are not left with any viable notion that MMC does not induce a fully loaded apoptotic response.

In summary, Rutherford et al have attributed to our report specific deficiencies that did not exist. If their initial intention was to reproduce our work, their group should have done the same experiments. In fact, they failed to reproduce conditions we used (TUNEL positivity is most substantial after 48 hours of exposure to IFN and agonistic fas antibody[2[Fig3]]), failed to use isogenic sets of FA mutant cells, and used a method for quantifying apoptotic cells that is not fully validated for studies on Fanconi cells, when compared to results from a battery of conventional measures of apoptosis, methods they themselves claim to be gold standards. Their concluding suggestion, "high constitutive levels of fas expression on FA cells" may account for some effects we have all seen, has been tested by us years ago and ruled out at least in B-cell lines.[4] When the authors test their next hypothesis, they should utilize isogenic FA cell lines now widely extant, use gold standard apoptosis assays, use the combinations of cytokines now known to induce maximal differential apoptosis in FA cells, and consider using caspase 3–activation assays that can detect biologically meaningful fractional changes in cell populations. If they do these things, we have no doubt they will find that FA-C cells exhibit exaggerated responses that are both statistically and biologically significant. We suppose that one might spend a good bit of time arguing about certain semantic issues that have evolved in this field, issues that might be likened to the tree-falling-in-the-forest-unseen argument (eg, if all other molecular events characteristic of apoptosis occur, does a reduced detectability of internucleosomally cleaved DNA mean that there is no "apoptosis?"). Moving beyond such arguments, we are confident that most workers in this field, including the 2 parties disagreeing here, will ultimately concur that FA-C cells are more poised to undergo programmed cell death when challenged with certain chemical agents or selected extracellular biologic cues and that the apoptotic response of these cells is

Downloaded from http://ashpublications.org/blood/article-pdf/99/7/2627/1682299/2627a.pdf by guest on 25 June 2024

generally exaggerated because the protein encoded by the FANCC gene modulates such responses.

Grover C. Bagby Jr

Correspondence: Grover C. Bagby Jr, Director, Oregon Cancer Center, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR 97201-3098

References

1. Rathbun RK, Christianson TA, Faulkner GR, et al. Interferon-g-induced apoptotic responses of Fanconi anemia group C hematopoietic progenitor cells involve caspase 8-dependent activation of caspase 3 family members. Blood. 2000;96:4204-4211.
2. Rathbun RK, Faulkner GR, Ostroski MH, et al. Inactivation of the Fanconi anemia group C (FAC) gene augments interferon-gamma-induced apoptotic responses in hematopoietic cells. Blood. 1997;90:974-985.
3. Fagerlie SR, Diaz J, Christianson TA, et al. Functional correction of FA-C cells with FANCC suppresses the expression of interferon gamma-inducible genes. Blood. 2001;97:3017-3024.
4. Koh PS, Hughes GC, Faulkner GR, Keeble WW, Bagby GC. The Fanconi anemia group C gene product modulates apoptotic responses to tumor necrosis factor-a and Fas ligand but does not suppress expression of receptors of the tumor necrosis factor receptor superfamily. Exp Hematol. 1999;27:1-8.
5. Pang Q, Fagerlie S, Christianson TA, et al. The Fanconi anemia protein FANCC binds to and facilitates the activation of STAT1 by gamma interferon and hematopoietic growth factors. Mol Cell Biol. 2000;20:4724-4735.
6. Pang Q, Keeble W, Diaz J, et al. Role of double-stranded RNA-dependent protein kinase in mediating hypersensitivity of Fanconi anemia complementation group C cells to interferon gamma, tumor necrosis factor-alpha, and double-stranded RNA. Blood. 2001;97:1644-1652.
7. Pang Q, Keeble W, Christianson TA, Faulkner GR, Bagby GC. FANCC interacts with hsp70 to protect hematopoietic cells from IFNg/TNFa-mediated cytotoxicity. EMBO J. 2001;20:4478-4489.
8. Pang Q, Christianson TA, Keeble W, et al. The Fanconi anemia complementation group C gene product: structural evidence of multifunctionality. Blood. 2001;98:1392-1401.
9. Wang Z-Q, Stingl L, Morrison C, et al. PARP is important for genomic stability but dispensable in apoptosis. Genes Dev. 1997;11:2347-2358.
10. Guillouf C, Wang TS, Liu J, et al. Fanconi anemia C protein acts at a switch between apoptosis and necrosis in mitomycin C-induced cell death. Exp Cell Res. 1999;246:384-394.
11. Clarke AA, Philpott NJ, Gordon-Smith EC, Rutherford TR. The sensitivity of Fanconi anaemia group C cells to apoptosis induced by mitomycin C is due to oxygen radical generation, not DNA crosslinking. Br J Haematol. 1997;96:240-247.

To the editor:

Epstein-Barr virus (EBV)–DNA quantification in pediatric allogeneic stem cell recipients: prediction of EBV-associated lymphoproliferative disease

Allogeneic hematopoietic stem cell transplantation (HSCT) using a graft from a donor other than an HLA-identical family member is increasingly used in the treatment of children and adults with malignant as well as nonmalignant diseases. To prevent the occurrence of serious graft-versus-host disease (GvHD) in these patients, T-lymphocyte depletion (TCD) of the graft is generally applied as an effective tool. In addition, immunosuppressive agents (eg, antithymocyte globulins [ATG] and fludarabin) are frequently included in preparative regimens. A major side effect of this approach is a prolonged period of severely compromised immune surveillance after HSCT. Consequently, this category of HSCT recipients has an increased risk of infections or reactivations of Epstein-Barr virus (EBV) and adenovirus associated with significant morbidity and mortality.

EBV infection, mostly of donor origin, may proceed to EBV-associated lymphoproliferative disease (EBV-LPD) in up to 15% of these high-risk patients, leading to death in the majority of them.[1] Several therapeutic strategies have been reported when clinical symptoms are present, including tapering of immune suppression and administration of anti–B lymphocyte monoclonal antibodies, all with limited efficacy.[2] Restoration of T-cellular immunity seemed to be the most powerful approach as clearly demonstrated by donor lymphocyte infusions (DLI) and most elegantly by administration of EBV-specific cytotoxic T lymphocytes (CTL).[3,4] Unfortunately, DLI may result in concomitant GvHD, whereas the generation of CTL is time consuming. Notably, early stage polyclonal or oligoclonal disease is more responsive to the already described therapeutic modalities compared to monoclonal disease. Therefore, the recognition of EBV reactivation at an early stage and prevention of progression to overt clinical disease and fulminant EBV-LPD is a major challenge in the management of these HSCT recipients. Early recognition of EBV reactivation at a molecular level by real-time quantitative polymerase chain reaction (RQ-PCR) to measure EBV-DNA load might be worthwhile in this perspective. However, only limited information is available concerning the applicability of this approach in clinical decision making.

Table 1. Patient characteristics

	T-cell–depleted SCT patients without EBV-LPD (n = 20)	T-cell–depleted SCT patients with EBV-LPD (n = 6)
Age, y		
Mean (range)	7.3 (1-16)	6.6 (1-13)
Diagnosis, n		
ALL CR1	3	
ALL CR2	4	1
AML CR1	1	1
FEL	2	1
SAA	1	2
Fanconi	1	
MLD	1	
X-ALD	2	
Hurler-Scheie	1	
SCID	1	1
JMML/CML	2	
MDS	1	
Donor type, n		
MUD	12	4
Haplo	8	2
Conditioning includes, n		
ATG	3	1
Campath-1H	2	1
ATG + anti-LFA-1	3	2
Campath-1H + anti-LFA-1	11	1
Positive EBV serology, n		
D/R	20/14	6/3
Stem cell graft preparation, n		
$CD34^+$ selection	7	2
T and B immunorosetting	12	3
Other*	1	1

ALL CR1 indicates acute lymphoblastic leukemia first remission; AML CR1, acute myeloblastic leukemia first remission; FEL, familial erythrophagocytic lymphhistiocytosis; SAA, severe aplastic leukemia; MLD, metachromatic leukodystrophy; X-ALD, X-linked adrenoleukodystrophy; JMML/CML, juvenile myelomonocytic leukemia/chronic myeloid leukemia; MDS, myelodysplastic syndrome; LFA-1, lymphocyte junction-associated antigen type 1; D/R, donor recipient.

*Campath-1G in the bag and E-rosetting, respectively.

Downloaded from http://ashpublications.org/blood/article-pdf/99/7/2627/1682299/2627a.pdf by guest on 25 June 2024