

Letter to the Editor

Severe cutaneous human papillomavirus infection associated with natural killer cell deficiency following stem cell transplantation for severe combined immunodeficiency

To the Editor:

Hematopoietic stem cell transplantation (HSCT) has significantly improved survival in severe combined immunodeficiency (SCID). Earlier identification and intervention with HSCT is associated with fewer short-term complications and improved long-term survival.¹ Long-term follow-up studies of post-transplant SCID patients document sustained T cell engraftment that is nonetheless complicated by respiratory disease, growth and neurodevelopmental impairment, and susceptibility to chronic infections.² Severe, chronic human papillomavirus (HPV) infections have been noted in subsets of SCID patients post-transplant.^{3,4} This has been confined to only those having γ c or JAK3 mutations. The etiology of severe HPV infections in these patients has not been clearly defined.

Intact T-cell-mediated immunity and natural killer (NK) cell function are imperative for host defense against HPV infection.^{5,6} Fifty percent of subjects with $T^+B^+NK^-$ SCID due to γ c or JAK3 genetic mutations were identified as having severe HPV infections years post-HSCT.³ No specific differences in transplant-specific or immune parameters were defined between the patients with and without HPV. While median NK cell counts were lower in SCID patients with γ c or JAK3 mutations as compared with other SCID phenotypes, there were no significant differences in NK cell counts between those with and without HPV. NK cell cytotoxic function, while depressed, did not correlate with HPV infection.

We performed a retrospective review of SCID patients (n = 65) treated at our institution between 1981 and 2012. Standard post-HSCT evaluation included T, B, and NK cell enumeration at 100 days, 6 months, and annually. NK cell functional assays were performed in patients with HPV disease. Sixty demonstrated donor engraftment. None had HPV exposure at delivery or any

HPV clinical disease prior to HSCT. $T^+B^+NK^-$ SCID phenotype distribution was present in 52% (31 of 60) of the children. These were comprised of 21 γ c, 3 JAK3, 2 CD3 δ , and 5 unknown molecular defects. Fifteen (48%) of these 31 patients received no (n = 14) or reduced intensity conditioning (n = 1), and all others (n = 16) received myeloablative conditioning pre-HSCT.

Six (19%) of the patients died, all within 12 months of transplant, and all 6 had serious infections at presentation. Six of the surviving patients developed severe cutaneous HPV infection (2 or more locations and resistant to >3 forms of treatment), but none had genital lesions or epidermodysplasia verruciformis. No patient with HPV disease had other chronic viral infections.

Long-term follow-up records were not available for data extraction in 5 patients, 2 of whom had evidence of HPV disease. Extensive medical record review was performed for surviving patients and was possible for 20 patients (16 γ c, 2 JAK3, and 2 CD3 δ).

Four patients had >10-year post-HSCT follow-up and chronic HPV infection (Table I, and example of HPV disease in Fig 1, A). All had excellent T cell engraftment, with normal T-cell function, and 75% had normal B-cell function, post-HSCT (see Table E1 in this article's [Online Repository at www.jacionline.org](http://www.jacionline.org)). None of the HPV-affected patients received pre-HSCT conditioning. Mean age at onset of warts was 7.4 ± 1.4 years post-HSCT. All SCID cases without HPV disease (n = 16; 12 γ c, 2 JAK3, 2 CD3 δ) received myeloablative conditioning, and mean follow-up was 6.5 years post-HSCT.

As expected, the pretransplant NK cell numbers were low, mean 38 ± 75 cells/mm³. In the HPV cohort, the absolute number of NK cells post-transplant remained low and did not change significantly from the pretransplant NK cell numbers (mean 34 ± 22 cells/mm³; $P = .9$). In contrast, patients without HPV disease (n = 16) had good NK cell restoration post-HSCT, with a mean absolute number of NK cells increasing to 209 ± 137 cells/mm³ ($P < .0001$). The difference in NK cell immune reconstitution between patients with and without HPV disease was significant ($P = .02$; Fig 1, B). When the non γ c and JAK3 patients

TABLE I. Demographic, clinical, and immunologic characteristics of 4 patients with severe HPV disease

	Patient 1	Patient 2	Patient 3	Patient 4
SCID phenotype	$T^+B^+NK^-$	$T^+B^+NK^-$	$T^+B^+NK^-$	$T^+B^+NK^-$
Specific diagnosis	γ c	γ c	γ c	γ c
Age at transplant (d)	22	120	14	219
Type of transplant	MMRD mother	MMRD mother	MRD sister	MRD sister
Pretransplant conditioning	None	None	None	None
T-cell depletion	None	None	None	None
Engraftment	Mixed (T^+B^-)	Mixed (T^+B^-)	Mixed (T^+B^-)	Mixed (T^+B^-)
T cell engraftment (%)	100	99	98	100
Time of onset of warts after transplant (y)	7.75	9.4	7	5.75
Location of warts	Face, trunk	Trunk, arms	Face, hands, feet, arms, legs	Face, hands, feet, arms, legs
Other long-term comorbidities	PLE, asthma, on immunoglobulin replacement	None	None	None
Most recent ALC cells/mm ³	1755	2014	1650	1482
Most recent CD3 cells/mm ³	1615 (92%)	1672 (83%)	1518 (92%)	1289 (87%)
Most recent CD4 cells/mm ³	562 (32%)	467 (23%)	726 (45%)	888 (60%)
Most recent CD19 cells/mm ³	123 (7%)	282 (14%)	99 (6%)	133 (9%)
Most recent IgG mg/dL	599	873	1139	1690

ALC, Absolute lymphocyte count; MRD, matched related transplant; MMRD, mismatched related transplant; PLE, protein losing enteropathy.

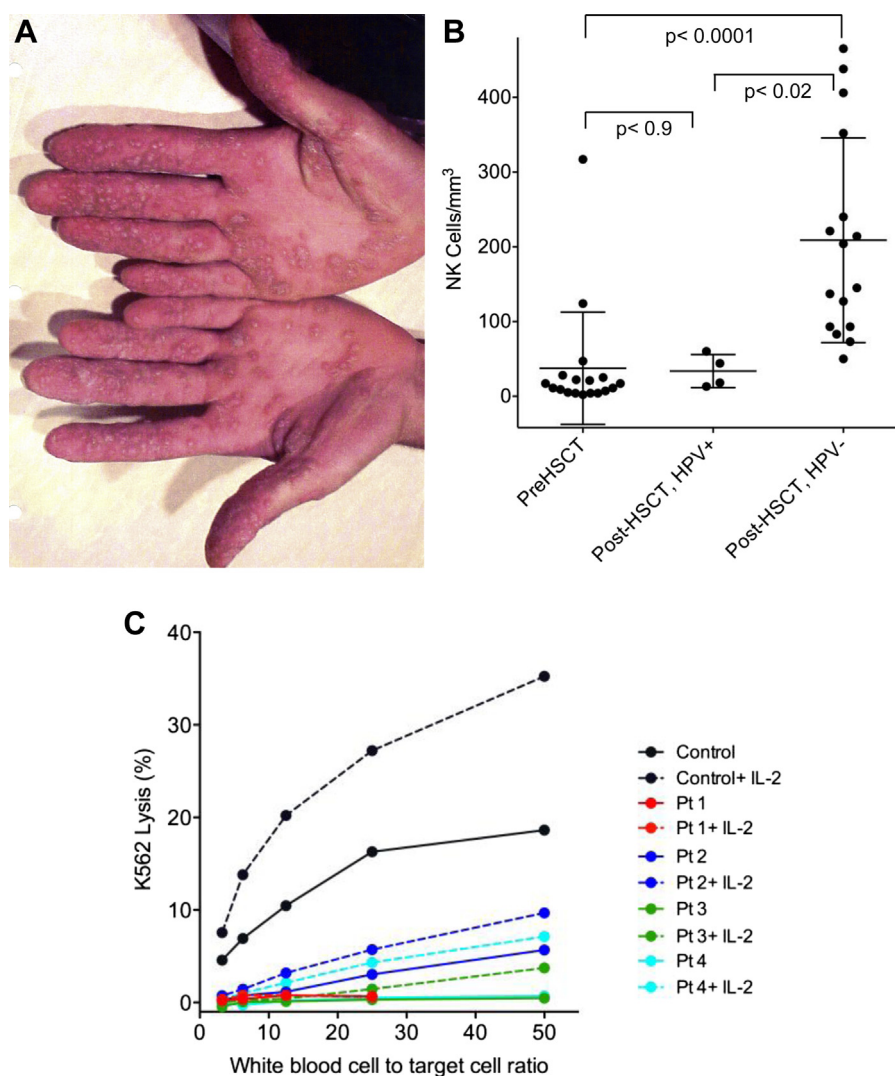


FIG 1. Multiple warts on the hands of a patient 7 years after HSCT for γ c SCID (**A**). HPV infection in post-HSCT patients is associated with low number of NK cells in PBMCs (**B**). NK cell numbers were compared in patients before (left) and after (middle and right) HSCT. Failure to increase NK cell number ($P < .09$) after transplant is noted in patients with HPV (middle), whereas patients without HPV show higher number of NK cells ($P < .0001$) (right). P values were obtained by 2-tailed t test. IL-2 rescues cytotoxic effect of NK cells in HPV-positive SCID patients (**C**). Four-hour cytotoxicity assay was performed against K562 target cells using PBMCs from healthy donors (black) and patients with HPV (red, blue, green, and teal) in the presence (dotted line) or absence (solid line) of IL-2 (1000 U/mL); increases in IL-2-mediated cytotoxicity were significant ($P < .002$; 2-way ANOVA).

were excluded from the analysis, the NK cell numbers were still significantly lower in patients with HPV disease (mean 34 ± 22 vs 175 ± 105 cells/mm³; $P = .02$).

In the HPV cohort, NK cell cytolytic activity was performed using PBMC freshly isolated from whole blood in chromium-51-release assays against K562 target cells in an effort to functionally confirm the reduction in NK cell counts.⁷ *In vitro* NK cell cytotoxic function was severely depressed in all 4 patients (Fig 1, C). Stimulation with IL-2 (1000 U/mL) modestly but significantly improved cytotoxic function. CD107a mobilization was measured by flow cytometry to assess the NK cell degranulation process in a single patient. Despite the low NK cell counts, the existing NK cells demonstrated abnormal degranulation, with $<50\%$ of normal mean channel fluorescence for induced CD107a levels.

Our study, consistent with previous reports, demonstrated chronic HPV disease in $T^+B^+NK^-$ SCID patients after HSCT. All $T^+B^+NK^-$ SCID patients without conditioning and with poor evidence of NK cell engraftment developed warts over 7 years of follow-up. In contrast to previously reported rates (50% to 64%) of severe HPV disease, our cohort showed a lower rate (19%) of HPV-affected children overall. While our numbers were relatively small, in contrast to other studies, we observed significantly depressed NK cell counts in patients with HPV disease. Orange et al have previously described improved *in vivo* and *ex vivo* NK cell function in Wiskott-Aldrich patients with IL-2 administration through F actin reorganization.⁸ We also noted that IL-2 was able to promote at least some cytotoxic activity from patient cells *in vitro*, suggesting a potential topic for further therapeutic research.

Study limitations include absence of data for 2 HPV patients who were lost to follow-up. We had a shorter follow-up period for HPV-negative T⁺B⁺NK⁺ SCID, 6.5 years versus >10 years for the HPV cohort. This could have introduced an ascertainment bias for HPV detection and may explain our lower overall rate of identified HPV. However, it is clear that post-HSCT, the cohort without HPV disease had markedly improved NK cell engraftment when compared with their counterparts with HPV disease.

Investigators have proposed that in γ c receptor deficiency, dendritic cells may remain dysfunctional post-HSCT and thus permissive to HPV infection despite donor T cell engraftment.³ No specific data supports this theory. Our study revealed a lower incidence of HPV disease than in other cohorts, likely related to the lower mean age of our HPV-negative cohort and excellent engraftment of T, B, and NK cells in our entire cohort. We propose that poor NK cell engraftment and function represents a more likely contributor to the development of severe HPV disease in our 4 patients. It is possible that pre-HSCT myeloablation improved NK cell engraftment and function, allowing for improved response to HPV exposure in later life and thus disease limitation. With improvements in early diagnosis and curative treatment in SCID, it is imperative to review and determine the etiology of long-term adverse events like severe HPV disease following transplant.

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TABLE E1. Immunologic variables in all patients post-HSCT

	HPV disease present (N = 4)	HPV disease absent (N = 16)
T cells <1000/ μ L	0/4	0/16
CD4 cells <300/ μ L	0/4	0/16
Abnormal lymphocyte proliferation	0/4	0/16
IgG supplementation	1/4	2/16