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Original article

Gamma Delta T Cells: A Prospective Analysis of Their Regeneration Kinetics and Their Impact on the Clinical Outcome Following Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Haematopoietic stem cell transplantation (HSCT) plays an important role in the therapy of hematological malignancies. Gamma delta T cells ($\gamma\delta$ T cells) are a distinct lineage of T lymphocytes that might play an important role in immune recovery and could utilize a graft –versus – leukemia effect post HSCT, furthermore, higher counts of $\gamma\delta$ T cells could improve clinical outcome after HSCT. This work was conducted to analyze the kinetics of gamma delta T cells recovery post HSCT, and to correlate their reconstitution with different factors that may influence the clinical outcome following HSCT. In this study, 22 consecutive allogeneic hematopoietic stem cell transplantation (HSCT) recipients were analysed during the first-year post transplantation by measuring the absolute count of CD3 T cells and percentages of gamma delta T cells subsets every month for each patient using flow cytometric technique. Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results were presented as mean values of CD3+, gamma delta T cells %, and p-values. Higher gamma delta T cells percentages were significantly correlated with younger patient and donor age, sex matched transplantation, leukemic diseases, un-manipulated transplants, and in patients without chronic graft versus host disease complications. Furthermore, positive correlation between CD3 T cells counts and gamma delta T cells % was also determined. Overall survival and better clinical outcome following allogeneic HSCT could be related with proper gamma delta T cells reconstitution.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is considered as a potential therapy for hematological malignancies in adults and pediatrics, because it provides a strong cure for a different of life threating hematological and non-

hematological disorders [1,2]. The major causes of post transplantation mortality are underlying disorder relapse, infections, and graft-versus-host disease (GVHD). The effective HSCT outcome was due to many factors including using of less toxic conditioning regimens and developing the understanding of the immune mechanisms [3,4]. Immune reconstitution after allogeneic Hematopoietic stem cell transplantation (HSCT) generates from the donor-derived progenitors and the proliferation of the immune cells transferred with the graft. There is a significant difference in the kinetics of innate and adaptive immune recovery and the rapid reconstitution of monocytes and natural killer (NK) cells comparing with the delayed maturation of T and B lymphocytes, which may not complete until the first year after transplantation. Reconstitution of functional immune responses affected by many factors, particularly source of graft, graft versus host disease and/or its preventive therapy. Complete and functional recovery of both innate and adaptive immunity is necessary to limit the susceptibility to infection and to prevent relapse risk after allogeneic HSCT [5]. Numerous reports have demonstrated that Long defence insufficiency following HSCT occurs mainly from deficiencies in effective cellular immune reconstitution [6-8]. Therefore, efficient recovery of a functional immune system is critical for successfully outcome [9]. Better understanding of T cell recovery kinetics and the elements affecting this procedure after allogeneic HSCT are important for better HSCT outcome [10].

Gamma delta T cells (γδ T cells) are a distinct lineage of T lymphocytes that can be characterized from conventional T cells through their receptors that involve γ and δ chains rather than α and β chains [11,12] and they have an important role in natural immunity against many infections and exert functional antitumor activity [13]. Recently, Comprehending the role of γδ T cells in HSCT has been the topic of different studies [14,15]. Early works found that long-term disease –free survival of leukemia patients who received αβ T-depleted partially mismatched related donor transplants had high levels of circulating γδ T cells post HSCT [16,17].

Gamma delta T cells also have a role in stress immune-control [18], tissue homeostasis, restoration of wounds, and heat-management [19, 20]. However, their detailed reconstitution after allogeneic HSCT remains poorly understood. The anti-viral ability of γδ T cells have been reported for various viruses such as Epstein Bar virus (EBV), Cytomegalovirus (CMV), influenza, hepatitis C virus, and more recently severe acute respiratory syndrome coronavirus 2 (SARS -COV-2) [21]. Gamma delta T cells react early to viral infections through up regulation of their Toll-like receptors which recognize pathogen-associated molecular patterns (PAMPs), such as viral molecules, initiating a cascade which leads to production of interferon and pro-inflammatory cytokines [22]. This work was conducted to analyze the kinetic of gamma delta T cells reconstitution following allogeneic haematopoitic stem cell transplantation (HSCT) and to correlate their recovery with different factors that affect their regeneration.

METHODS

A prospective observational study was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany in the period between January 2009 to January 2010. About 22 consecutive patients (both male and females) of an age between 0.5- 26 years were included in this study. The sample size was estimated on the basis of a single proportion design.

After written informed consent was obtained, fresh whole blood specimens were collected once on day 30, day 60, day 90, day 120, day 150, day 180, day 210, day 240, day 270, day 300, day 330, and day 360 post transplantation The study protocol was approved by laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany. In patients underwent reduced-intensity conditioning transplantation, total donor chimerism was assessed from bone marrow aspirates. Genotyping was analysed by short tandem repeat typing using the ABI 310 Genetic Analyser (Applied Bio systems, Inc., Foster City, CA). Alleles specific to donor or recipient were used for chimerism identification.

Patients and transplant characteristics are presented in (Table 1). All patients received Cyclosporine A as GVHD prophylaxis, with either mycophenolate mofetil or Methotrexate. Graft versus-host disease (GVHD) was defined as acute if it occurred before day 100 and chronic thereafter. Lymphocytes subsets were analyzed using four-color FACS CAN (BD, USA) flow cytometer. T cells were detected using Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (clone SK 7), and Phycoerythrin (PE)-conjugated anti-TCR γδ(clone 1IF2).

Aliquots of 50 microliter of Ethylene diaminetetraacetic acid (EDTA)or heparin blood were placed in FACS tubes (BD, USA) and stained with the appropriate antibodies (titrated for optimal concentration), then incubated shortly in the dark place. Finally, the erythrocytes were lysed, washed, and FACS-lysing solution; (BD Pharmingen, USA) was added for the final fixation. Cells were analyzed on FACS CAN (BD, USA) flow cytometer. Data was further analyzed using Cell Quest program software. $CD3^+$ T cells for every patient were gated and quantified every month from day 30 until day 360 post transplant. γδ T cells were presented as a percentage of total CD3+ T cell count.

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results are presented as mean values of Lymphocytes, % of gamma delta T cells, and *p*-values. Data was analyzed using the

Licensed IBM SPSS 20.0 for Windows statistical analysis packages. Student's *t*-test was used to ascertain the significance of differences between mean values of two continuous variables and confirmed by nonparametric Mann-Whitney test. The differences in the indicators were considered statistically significant at $p \leq 0.05$.

Table 1. Patients and transplant characteristics

Characteristics	Number (%)
Males Females	14(64%) 8(36%)
Patients 'age mean(range)	10.8(0.5-26)
Donor age mean (range)	30(7-50)
Stem cell source Peripheral blood Bone marrow	 5(23%) 17(77%)
T cell- repletion	2(9%) 1x 10^6cells
Acute GVHD (grade I-II) Chronic GVHD	20(91%) 16 (73%)
Donor code Matched related donor (MRD) Matched unrelated donor (MUD)	 5(23%) 17(77%)
HLA typing 10/10 9/10 8/10	 15(68%) 4(18%) 3(14%)
Hematological disease Malignant Non-malignant	13(59%) 9(41%)
Immunosuppression ATG Post-Cy both	 16(73%) 12(55%) 7(23%)

GVHD= Graft versus-host disease, HLA= Human leukocyte antigens, ATG=anti-thymocytglobuin, Post-Cy= post- transplant cyclophosphamide

RESULTS

Reconstitution of Lymphocytes

To analyze the lymphocytes-subset reconstitution kinetics in all 22 patients, absolute count of $CD3^+$ lymphocytes/µl was measured in whole blood every month during first year of follow- up. Counts of $CD3^+$ lymphocytes and gamma delta T cells (%) were measured and presented as mean, minimal, and maximal values (Table 2&3)

Table 2. CD3+ lymphocytes /µl during 12months post allogeneic HSCT

Time post HSCT	Mean	Minimum	Maximum
Day 30	529	39	1687
Day 60	619	42	1773
Day 90	708	80	1916
Day 120	831	81	2215
Day 150	963	145	3100
Day 180	1258	150	4409
Day 210	1312	163	5900
Day 240	1521	173	7026
Day 270	1726	181	7134
Day 300	1819	185	7254
Day 330	1915	198	8111
Day 360	1997	232	8235

Table 3. γδ T cells % during 12months post allogeneic HSCT (Mean, Minimal-/ Maximal value

Time post HSCT	Mean	Minimum	Maximum
Day 30	3	0	12
Day 60	5	0	15
Day 90	6	0	25
Day 120	7	0	34
Day 150	8	0	35
Day 180	9	0	48
Day 210	10	1	48
Day 240	11	2	50
Day 270	11	2	48
Day 300	11	2	48
Day 330	11	2	47
Day 360	12	3	46

Impact of Patient age on the γδ T cells recovery

The recovery of γδ T cells over time was significantly higher in patients younger than 15 years($P \leq 0.001$) (Fig.1)

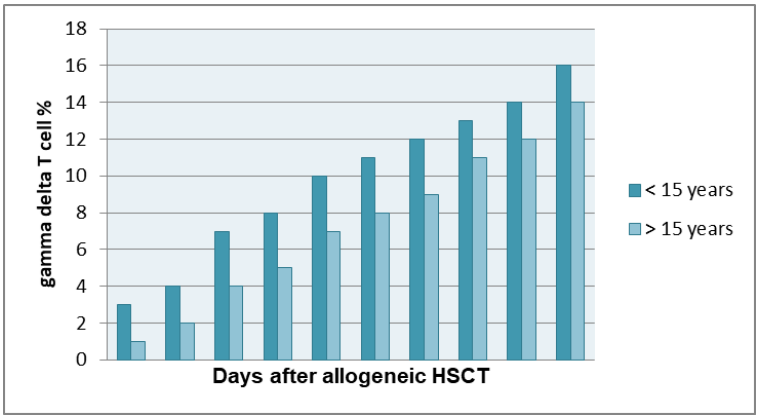

Figure 1. Impact of patient age on γδ T cells recovery

Impact of Donor age on the γδ T cells recovery

The recovery of γδ T cells over time was significantly higher in recipients from donors younger than 30 years (P= 0.002) (Fig 2).

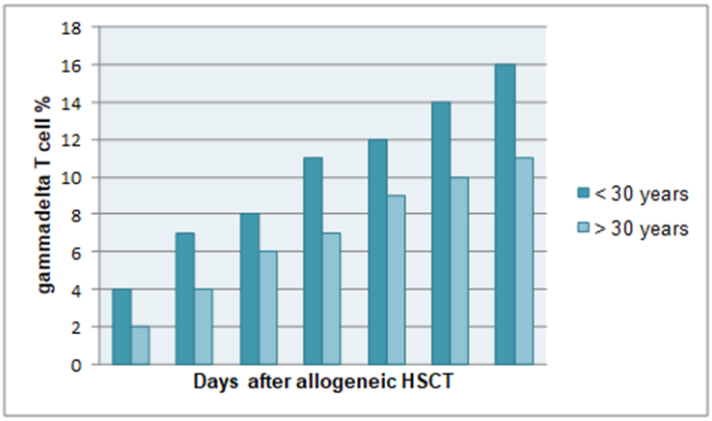

Figure 2. Impact of donor age on γδ T cells recovery

Impact of viral infections on the γδ T cells recovery

Viral infections/reactivations post allo-HSCT include :64% cytomegalovirus (CMV),91%Epstein- Barr virus (EBV),73% adenovirus,36% human herpes virus 6(HHV6), 23%herpes simplex virus,18% Norovirus, 27%BK virus,23% hepatitis G virus,27% varicella zoster,9% influenza A,6% influenza B,9% Parvovirus B19,14% Rota

Virus,18% Enterovirus, 6%Polymavirus,9% Rhinovirus,18% Para influenza 1,2,3,9% Respiratory syncytial virus (RSV), and9% measles. Concerning with the prospective analysis of role of gamma delta T cells in several viral infections, we divided patients based on the viral reactivation into: patients without viral infections/reactivation 9%(n=2); one viral infection/reactivation 23%(n=5); two viral infections/reactivation 18%(n=4); three viral infections/reactivation 14%(n=3); more than three viruses 36% (n=8). Percentages of gamma delta T cells following allo- HSCT showed significantly higher values in patients with multiple viral reactivation *P*=0.001) (Fig.3).

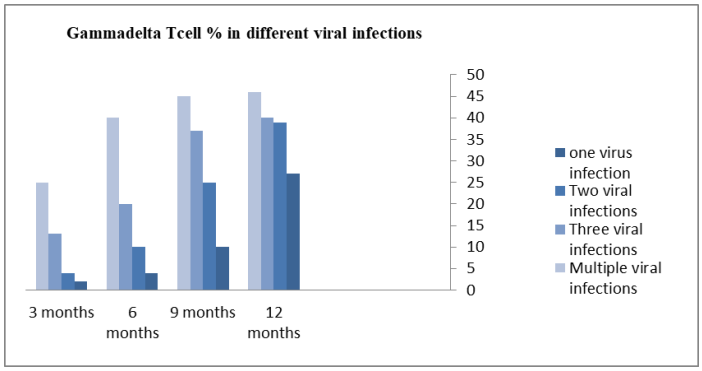

Figure 3. Impact of virus infections on γδ T cells recovery

While acute GVHD had no effect on their reconstitution, $CD3^+$ T cells counts (Fig.4) and gamma delta T cells% were also significantly higher in patient without chronic graft versus disease(cGVHD) (*P*=0.002) (Fig.5).

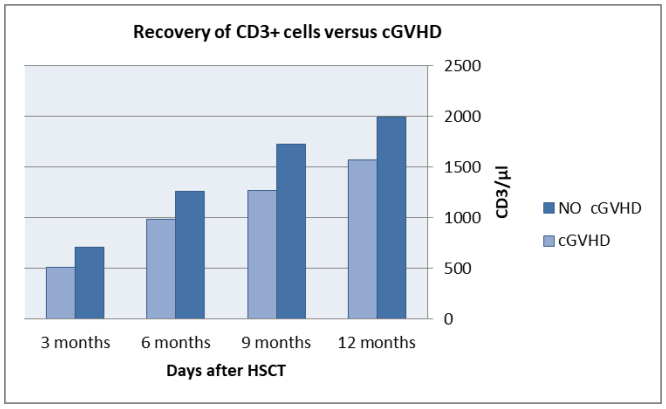

Figure 4. Impact of cGVHD on CD3 T cells recovery

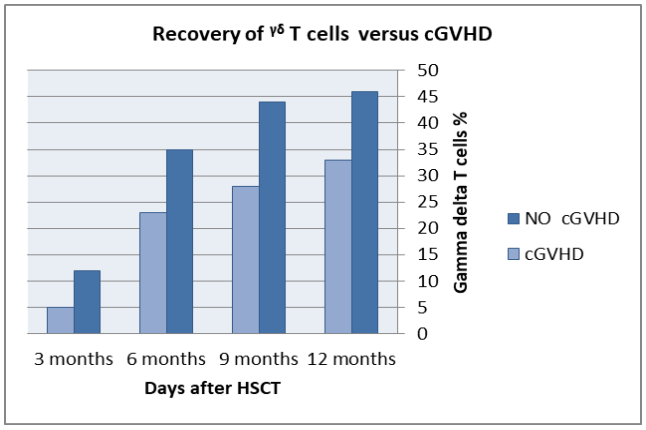

Figure 5. Impact of cGVHD on T cells recovery

Univariate analysis of elements influencing recovery of γδ T cells

We studied the association between recovery of γδ T cells and several factors following allo-HSCT and found that their reconstitution was affected significantly by several factors following allo-HSCT.

Table 4. General factors influencing recovery of γδ T cells

Gamma delta T cell recovery	P value
Patient age:< 15 years versus > 15	≤ 0.001
Donor age: <30 years versus > 30	0.002
Chronic GVHD: no versus yes	0.002
Underlying disease: Malignant versus non-malignant	≤0.03
Acute GVHD: no versus yes	0.40
Sex match: match versus mismatch	0.002
Graft manipulation: CD34+ selected Peripheral blood versus un-manipulated Bone marrow	0.0001
Relation with CD3+ recovery	0.003
One virus reactivation	0.13
Two virus reactivations	0.26
Three virus reactivations	0.02
Multiple viral infections	0.001

DISCUSSION

Regeneration of leukocytes following allo-HSCT differs from one cell type to another. In general, cells of natural immunity reconstitute earlier, while the recovery of acquired immunity is more prolonged [10, 23]. Only a few studies have focused on the recovery of γδT cells following allo-HSCT and proved that γδ T cells regeneration occurs in the first few weeks after transplantation [15, 21, 22, and 24].

Many previous studies suggested that γδT cells recover early and gradually decrease as αβ T cells begin to recover [24-26]. Enhanced immune reconstitution of $^{\gamma\delta}$ T cells suggests that they originate from peripheral expansion of donor gamma delta T cells infused within the grafts [26]. Our study showed that younger patient and donor age, sex match, and high CD3+ cell numbers correlated positively with the recovery of $^{\gamma\delta}$ T cells and associated with faster gamma delta T cells recovery [27].

As previous reports, we found that reconstitution of gamma delta T cells was slower in recipients of CD34+selected peripheral stem cell transplants compared to recipients of un-manipulated grafts.[27]. But it is difficult to deduce whether this occurs due to the graft source or due to the manipulation effect of peripheral stem cell transplants.

Graft versus host disease (GVHD) is considered as one of the major dangerous complications of allogeneic HSCT. GVHD is divided into acute GVHD and chronic GVHD depending on the time and type of organ involvement. Several studies have reported that higher γδT cells count was associated with less risk of acute GVHD. However, according to our results, the percentages of gamma delta T cells in non-chronic GVHD (cGVHD) group were significantly higher than those with cGVHD complications. As previous reported the elevated number of γδT cells was associated with prevention and controlling chronic GVHD after allo- HSCT [28]. Additionally, higher gamma delta T cell percentages were also detected in malignant patients and that can be related to their potential role against leukemic cells and preventing relapse and was associated with better outcome after allo-HSCT [28, 29].

Regarding the anti-viral capacities of gamma delta T cells, it was described that they are able to react early against viral infection by up regulation of Toll like receptor to recognize viral particles and also through expressing natural killer type receptors which can activated against virally infected cells by supporting cytotoxicity and also inducing apoptosis of virally infected cells. Analysis of the role of gamma delta T cells in viral infections, displayed higher values in patients with multiple viral reactivations. While most studies of the function of γδT cells against viral infections or reactivation following allo-HSCT are focused on herpes viruses such as CMV or EBV, studies concerning their capacities against other herpes viruses such as on human herpes virus 6 (HHV6) and varicella-zoster , or non-herpes viruses such as adenovirus, and BK virus are limited. Reports on human herpes virus 8 (HHV8) showed that during infection, an elevation in some gamma delta subset count is detected [30]. StimulatedγδT cells were able to destroy influenza –infected lung epithelial cells in vitro in addition to their capacity of virus clearance from the site of infection [31]. In general, viral infections permanently change the composition and phenotype of γδ T cells and their exact function in viral infection in relation to other immune components remain to be illustrated.

CONCLUSION

Gamma delta T cells ($^{\gamma\delta}$ T cells) are a distinct lineage of T lymphocytes that might play an important role in immune recovery and could utilize a graft –versus –leukemia effect following HSCT. Our experimental results have found that

overall survival and better clinical outcome following allogeneic HSCT could be related with proper gamma delta T cells reconstitution.

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Conflicts of Interest
The author declares no competing interests.

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خلايا جاما دلتا التائية: تحليل مستقبلي لحركية تجديدها وتأثيرها على النتائج السريرية بعد زرع الخلايا الجذعية المكونة للدم الخيفي

عيدة المنصوري

قسم علوم المختبرات الطبية، كلية التقنية الطبية، جامعة طرابلس، ليبيا

المستخلص

زراعة الخلية الجذعية الدموية تلعب دورا اساسيا في علاج الامراض السرطانية التي تصيب الدم. الخلايا الليمفاوية من نوع جاما- دلتا وهي نوع متميز من الخلايا الليمفاوية التائية التي لها دور في اعادة تشكل المناعة بعد الزراعة ,كما ان لها دورا في القضاء على الخلايا السرطانية التي قد تنشط بعد الزراعة بمعنى اخر ان زيادة عدد خلايا جاما-دلتا ,قد يرافقها تحسن الحالة السريرية للمرضى بعد الزراعة. هدف البحث: تم اجراء هذا البحث لتحليل الية تطور خلايا جاما-دلتا بعد الزراعة, و ربط تطورها بالعديد من العوامل التي قد تؤثر على الحالة السريرية للمرضى بعد الزراعة. تم تحليل عينات من 22 مستقبلا لزراعة الخلية الجذعية الدموية خلال العام الاول من الزراعة, وذلك بقياس العدد المطلق للخلايا التائية الحاملة للمستقبل سي دي 3 وكذلك نسبة خلايا جاما-دلتا كل شهر باستعمال طريقة التدفق الخلوي وتحديد المتوسط, القيمة الاعلى والقيمة الادنى. وجدنا ان النسبة المرتفعة لخلايا جاما- دلتا ترتبط مع العمر الصغير للمريض والمتبرع, وكذلك عند تطابق الجنسين بين المتبرع والمستقبل, وفي مرض سرطان الدم الابيضاضي, وفي الزراعة غير المعالجة, وكذلك عند المرضى بدون المضاعفات الناتجة عن تفاعل الخلايا المزروعة مع خلايا المستقبل النوع المزمن, وبالإضافة الى ذلك تم ايجاد علاقة ايجابية بين نسبة خلايا جاما- دلتا وعدد الخلايا التائية الحاملة للمستقبل سي دي 3. بشكل عام, القدرة على البقاء وتحسن الحالة السريرية للمرضى بعد زراعة الخلايا الجذعية الدموية له علاقة بتحسن اعادة تشكل خلايا جاما-دلتا بعد الزراعة.

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