Cytokine Production by Graft Cells in Response to Patient's Antigens May Predict the Occurrence of Acute Graft Versus Host Disease in Allogeneic Hematopoietic Stem Cell Transplantation from a Sibling

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INTRODUCTION & OBJECTIVES

Introduction:
Allogeneic hematopoietic stem cell transplantation (HSCT) whether bone marrow (BM) or peripheral blood (PBSCST) is a widely used therapeutic modality. Graft versus host disease (GVHD) continues to be the main concern of transplanters. There is substantial evidence to implicate that cytokines play a major role in GVHD induction and grade. GVHD was reported to be associated with increased production of IFNγ and IL10. IL10 level and gene polymorphism have been used as predictors of acute GVHD (aGVHD). IFNγ was also reported to have a protective role if administered at the time of transplantation possibly via induction of T regulatory cells and immunological tolerance.

Objectives:
(1) To develop an experimental setup to mimic the response of the immune cells of the graft to the host antigens expressed by cytokine production.
(2) To correlate the cytokine pattern to the development of aGVHD.
(3) To verify if this pattern could possibly predict the occurrence of aGVHD.

PATIENTS & METHODS

Patients:
The study included 46 patients who received allogeneic PBSCST from an identical sibling at Nasser Institute in the period from November 2004 until May 2008. They included 31 male and 26 female. Their age ranged from 6 to 41 years old with a mean of 22.3 ± 9.39 and a median of 22 years.

Methods:
Under informed consent, a sample was obtained from the patient before conditioning, mononuclear cells separated and cryopreserved.

On the day of transplant, the cryopreserved cells were thawed, mitomycin treated to serve as stimulators while the mononuclear cells of the graft served as the responders in a mixed lymphocyte culture setup. After 3 days culture at 37°C, 5% CO2, the culture supernatant was collected and stored at -80°C till tested.

IFNγ and IL10 were measured by microbead array technology using luminex 200 and Fluorokine MAP kit provided by R&D Company (Human multianalyte profiling base kit A and Human multianalyte profiling base kit B).

Patients were followed up for, at least, one year and development of aGVHD was recorded.

RESULTS

Of the 46 patients, 14 developed aGVHD. In the culture supernatant, cytokines were below the detection limit in 26/32 of cases that did not and in 3/14 of those who developed aGVHD. The level of cytokines in the other cases varied widely. Of the 11 cases that showed cytokine production, 4 produced IFNγ only and one produced IL10 only. The other 6 cases produced both: IL10 was higher in one case; in the other 5 cases, IFNγ was much higher. In the 8/32 cases without GVHD, IL10 only was detected in 3 cases; the other 3 cases showed much higher level of IL10 than IFNγ. The levels of IFNγ and IL10 in culture supernatant are presented in table 1. Acute GVHD was associated with significantly higher levels of both cytokines. Table 2 presents the frequency of cytokine production by the graft immune cells in culture and the level of the produced cytokines in relation to aGVHD. Significantly higher % of patients who developed aGVHD was cytokine producers; the cytokines produced were significantly higher than that produced by patients who did not develop aGVHD. The value of IFNγ and IL10 and IFNγ/IL10 to predict the occurrence of aGVHD is presented in table 3 and fig 1. When both IFNγ and IL10 are produced, IFNγ/IL10 ratio is superior to either IFNγ or IL10 alone in prediction of aGVHD.

CONCLUSIONS

- In vitro cytokine production by graft immune cells in response to host antigens is extremely variable.
- It may serve as a surrogate system of the immune reaction following allogeneic stem cell transplantation.
- IFNγ production apparently reflects potential development of aGVHD while IL10 production is apparently protective.
- When both are produced the IFNγ/IL10 ratio is more informative than either alone.

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References


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