Cutting Edge Communication

Interleukin-18 Binding Protein in Acute Graft versus Host Disease and Engraftment Following Allogeneic Peripheral Blood Stem Cell Transplants

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ABSTRACT

Dysregulation of the cytokine network plays an important role in graft-versus-host disease (GVHD). Interleukin-18 (IL-18) is an obligatory cytokine for interferon-γ (IFN-γ) production and IFN-γ and sIFN-γR are elevated in patients with GVHD. Because IL-18 binding protein (IL-18BP) is an inhibitor of IL-18-mediated IFN-γ production, we evaluated IL-18BP levels in patients undergoing allogeneic peripheral blood stem cell transplantation (PBSCT). IL-18BP levels were assessed in 14 patients on day −10 (before conditioning), on the day of transplant, on the day of engraftment, and during transplant-related complications. A comparison of the kinetics of IL-18BP and soluble(s) IL-6R, sIFN-γR, IL-18 serum levels was performed. IL-18BP levels were assessed by specific monoclonal antibodies in a double-sandwich enzyme-linked immunosorbent assay (ELISA). In all patients IL-18BP levels decreased during conditioning and increased in parallel with engraftment (p < 0.05). Accordingly, during rejection, IL-18BP serum levels remained low and similar to pretransplant levels. The mean elevation of IL-18BP detected in association to acute GVHD was significantly higher in comparison to normal engraftment (p < 0.05). A correlation between IL-18BP, sIFN-γR, and sIL-6R serum levels was found in all patients. No correlation between IL-18 and IL-18BP serum levels was found in patients undergoing uneventful PBSCT and rejection, whereas a marked increase in both IL-18 and IL-18BP levels was detected during acute GVHD (p < 0.01). Our data suggest that the dysregulation of IL-18 and IL-18BP may be important in the pathophysiology of transplant-related complications. Furthermore, because preliminary data from our group show that IL-18 blockade ameliorates GVHD in murine models, it is inferred that these cytokines may represent potential targets in the development of new therapeutic strategies in acute GVHD.

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INTRODUCTION

GRRAFT-VERSUS-HOST DISEASE (GVHD) and allograft rejection still represent major complications following allogeneic stem cell transplant (SCT). Further elucidation of their pathophysiological mechanisms is therefore of critical importance. Increasing evidence in experimental and clinical settings suggests that aberrant cytokine production occurs both in GVHD and graft rejection.

As currently proposed, it is assumed that acute GVHD develops as a three-step process characterized by sequential activation of monocytes and T cells as well as by aberrant cytokine production (1,2). In phase 1, recipient conditioning leads to host tissue injury and secretion...
of a variety of inflammatory cytokines, mainly tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, resulting in up-regulation of histocompatibility antigens and adhesion molecules on host target cells. Phase 2 is characterized by donor T cell activation and T-helper-type1 (Th1)-cytokine secretion, mainly IL-2 and interferon-γ (IFN-γ). In phase 3, Th1-derived cytokines lead to a synergistic host-donor cytokine reaction cycle: the subsequent “cytokine storm” mediates the amplification of local tissue injury and the destruction of target tissues through a cascade of multiple effectors, including mononuclear phagocytes, cytotoxic T lymphocytes (CTL), natural killer cells (NK), and several inflammatory cytokines (3,4).

Several lines of evidence have shown that IFN-γ plays a pivotal role in acute GVHD and is the predominant cytokine in TH1-dominated immune reactions (5). IFN-γ is secreted from T cells and NK cells and regulates a variety of immunological responses in both innate and acquired immunity (5,6). Increased serum levels of IFN-γ as well as soluble IFN-γ-receptor (sIFN-γR) and type-1 cytokines have been detected in association with acute GVHD (7).

Recent data also show that IL-18 is an obligatory cytokine for IFN-γ production in T cells and NK cells (8). IL-18, a novel macrophage-like cell-derived cytokine, acts in synergy with IL-12 in inducing IFN-γ production (9). IL-18 also enhances NK activity and together with IL-12 is thought to be a strong inducer of Th1 cell development (10). Increased serum levels of IL-18 have been detected during Th1-mediated chronic inflammatory disorders, such as Crohn disease (11,12) and hemophagocytic lymphohistiocytosis (13). By enhancing IFN-γ production, IL-18 may play a unique role in tumor immune surveillance and tissue homeostasis. In accordance, decreased synthesis of IL-18 has been observed in colon adenocarcinomas (14).

An IL-18-binding protein (IL-18BP) has recently been cloned by the group of Novick and Rubinstein (15), and its gene has been localized on chromosome 11q13 (16). IL-18BP is a member of a novel family of secreted proteins that belongs to the immunoglobulin (Ig) superfamily and also includes several poxvirus-encoded proteins. IL-18BP specifically binds IL-18 and neutralizes its biological activity in vitro and in vivo, thereby acting as a soluble decoy receptor (15). Because IL-18 is an early stimulant of TH1 cells, IL-18BP presumably plays an important role in the regulation of Th1-cytokine responses.

Although the cellular basis of the host-versus-graft response (i.e., rejection) has not been as well characterized, cytokines are thought to play a central role in engraftment and allograft rejection (17). It is a matter of fact that increased levels of inflammatory cytokines (mainly TNF-α, IL-6, and sIFN-γR) have been detected during both organ rejection and bone marrow graft rejection (18) and that sIL-6R and sIFN-γR serum levels display a positive correlation with engraftment in uneventful allogeneic transplants. On the basis of these considerations, we addressed the role of IL-18BP in acute GVHD and engraftment following allogeneic peripheral blood stem cell transplantation (PBSCT).

**MATERIALS AND METHODS**

*Patients’ characteristics*

Fourteen consenting patients (6 females and 8 males; mean age 26, range 5–51 years) who underwent unma-
Manipulated allogeneic PBSCT were enrolled in the study. Twelve normal individuals of compatible sex and age (and 9 patients who underwent uneventful SCT) served as controls. Most of the patients received pretransplantation our standard low-intensity conditioning which includes: Fludarabine 30 mg/m$^2$ for 6 days, Busulfan, 4 mg/kg for 2 days, and ATG (Fresenius) 10 mg/day for 4 days.

Methods

IL-18BP serum levels were determined in each patient and correlated to IL-18, sIL-6R, and sIFN-γR levels as well as to clinical parameters. IL-18BP, sIL-6R, and sIFN-γR levels were assessed by specific monoclonal antibodies in a double-sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (18). IL-18 levels were determined by sensitive radioimmunoassay (RIA).

IL-18BP serum levels were analyzed at day −10, at day 0, and at day of engraftment following PBSCT as well as during transplant-related complications including GVHD and graft rejection (GR). Engraftment was defined as day following transplant of white blood cells (WBC) $\geq 1 \times 10^9$/liter and absolute neutrophil count $0.5 \times 10^9$/liter. Graft rejection was defined as marrow hypoplasia (proven by bone marrow aspiration) with peripheral WBC $< 0.5 \times 10^9$/liter, 21 days after transplant, in conjunction with the absence of donor cells by the polymerase chain reaction

![Graph](image-url)

**FIG. 4.** Correlation between IL-18BP (unbroken lines) and sIFN-γR (a) and sIL-6R levels (b) (dashed lines) serum levels in engrafting patients. Pre, Day −10 pre-SCT; SCT, day 0 of SCT; Eng, day of engraftment (as defined in Patients and Methods) ($n=6$) (mean ± SE).
(PCR) using sex-mismatched-specific probes, microsatellite analysis, or ABO markers for engraftment.

The statistical analysis was performed using Student's two-tailed t-test for differences in the means.

RESULTS

In the patients undergoing PBSCT, 6 had an uneventful PBSCT whereas 5 developed acute GVHD and 3 had early GR. IL-18-BP levels in normal controls were 2.5 ± 2 ng/ml.

In the group of patients undergoing uncomplicated PBSCT, IL-18BP levels decreased during conditioning (6 ± 1 ng/ml at day −10 to 2.9 ± 0.8 ng/ml at day 0; p < 0.05) and then increased in parallel with engraftment. (10.5 ± 1.7 ng/ml at day of engraftment; p < 0.05) (Fig. 1). A similar trend toward a reduction of IL-18BP serum levels during conditioning was found both in patients undergoing GR (Fig. 2) (4.5 ± 1 ng/ml at day −10 to 1.8 ± 0.8 ng/ml at day 0) and in the subjects with acute GVHD (Fig. 3) (5.2 ± 0.9 at day −10, to 2.5 ± 0.7 at day 0; p < 0.01).

Whereas IL-18BP levels decreased in all patients during conditioning and then increased in parallel with engraftment, in the patients undergoing GR, post-transplant IL-18BP levels remained low and similar to pretransplant levels (3.5 ± 2.03 ng/ml) (Fig. 2). Furthermore, the mean

![Graph 1](image1.png)

![Graph 2](image2.png)

**FIG. 5.** Correlation between IL-18BP (unbroken lines) and sIFN-γR (a) and sIL-6R (b) (dashed lines) serum levels in patients with acute GVHD (as defined in Patients and Methods). Pre, Day −10 pre-SCT; SCT, day 0 of SCT; acute GVHD, day of overt GVHD (n = 5) (mean ± SE).
elevation of IL-18BP serum levels detected in the patients with acute GVHD (15 ± 2.5 ng/ml; \( p < 0.01 \)) (Fig. 3) was remarkably higher than the mean elevation observed in normal engraftment. IL-18BP levels were then related to sIFN-\( \gamma \)-R and sIL-6R levels. A correlation between IL-18BP, sIFN-\( \gamma \)-R, and sIL-6R serum levels was found both in the 6 patients undergoing uneventful (SCT) (Fig. 4) and in the 5 patients with acute GVHD (Fig. 5), because these cytokines displayed the same trend toward a reduction during conditioning and toward an increase in parallel with engraftment. IL-18BP levels also correlate with sIFN-\( \gamma \)-R and sIL-6R during rejection (Fig. 6) when the levels of these cytokines remained low and similar to pretransplant levels.

Because IL-18BP is known to be an inhibitor of IL-18-mediated IFN-\( \gamma \) production, IL-18 serum levels were also assessed to correlate IL-18BP and IL-18 production in uneventful transplant and during SCT-related complications. Unlike IL-18BP levels, no correlation between IL-18 serum levels and engraftment was found in patients undergoing uneventful PBSCT (Fig. 1) because IL-18 levels were similar at preconditioning (98 ± 9 pg/ml), at day 0 (83 ± 5 pg/ml), and at day of engraftment (115 ± 21 pg/ml). Accordingly, no significant difference between pre- and post-transplant IL-18 levels was observed during rejection (97 ± 9 pg/ml at day −10, 86 ± 5 pg/ml at day 0, 80 ± 6 pg/ml at day 21) (Fig. 2). By contrast, a marked increase in IL-18 levels (300 ± 29 pg/ml; \( p < 0.01 \)), paralleling the kinetics of IL-18BP levels, was detected in association with acute GVHD (Fig. 3). Basic disease, conditioning regimen, GVHD prophylaxis, and
grading had no effect on IL-18BP as well as IL-18, sIFN-γR, and sIL-6R levels (data not shown).

**DISCUSSION**

Dysregulation of the cytokine network has been shown to play an important role in the induction and maintenance of GVHD in experimental models and humans. In particular, an increasing amount of evidence has shown that IFN-γ is the predominant cytokine during Th1-dominated immune reactions and that cytokines and their soluble counterparts (which may modulate the biological activity of their respective cytokines) play a central role in the pathogenesis of both acute GVHD and graft rejection. In agreement with these observations, we have previously demonstrated that IFN-γ, sIFN-γR, and Th-1 cytokines serum levels are elevated in acute GVHD and that an increase in IFN-γ, sIFN-γR, and sIL-6R serum levels can be detected in parallel with engraftment in uneventful PBSCT (2,7,17,18). By enhancing IFN-γ secretion, IL-18 is thought to be a pivotal mediator in Th1-mediated responses.

IL-18BP is a recently identified secreted protein that specifically neutralizes IL-18-mediated IFN-γ production (15). Therefore, IL-18BP is suspected to play a central role in the regulation of Th1 cytokine responses.

On the basis of these assumptions and to address the role of IL-18BP in transplant-related complications, we studied the kinetics of IL-18BP serum levels in patients undergoing allogeneic PBSCT. Because previous observations showed a positive correlation between engraftment and sIFN-γR and sIL-6R levels, IL-18BP serum levels were then related to the kinetics of these cytokine-soluble receptors as well as to IL-18 levels.

We observed that IL-18BP levels declined during conditioning and then increased in direct correlation with engraftment both in uneventful and complicated SCT (Figs. 1–3). Furthermore, we showed that kinetics of IL-18BP correlated with sIL-6R and sIFN-γR levels and engraftment.

These findings confirm our previous published results showing that an elevation of sIL-6R levels is associated with engraftment and that hematopoietic regeneration is associated with increased sIFN-γR levels. The direct correlation between sIL-6R levels and engraftment is probably due to the fact that sIFN-γR and sIL-6R are secreted by the donor engrafting T cells. Therefore, it is conceivable that IL-18BP is also secreted by the engrafted mononuclear cells.

In this study, both IL-18 and IL-18BP serum levels reached higher values in the subgroup of patients developing acute GVHD (Fig. 3). The increase of IL-18 serum
levels was strikingly high and statistically significant (p < 0.05).

Because IL-18 is known to be a stronger inducer of IFN-γ secretion (8,9), it may be suggested that IL-18 promotes IFN-γ and sIFN-γR secretion by residual host T lymphocytes during engraftment. Thus, IL-18 may be the mediating factor leading to the increase of IFN-γ and IFN-γR levels that is associated with acute GVHD (5–7). IFN-γ may then give rise to negative feedback, which limits the Th1 response (Fig. 7). In accordance with this hypothesis, IFN-γ promotes IL-18BP secretion and IL-18BP in turn down-regulates IL-18 production. Indeed we have preliminary data showing that IL-18 blockage ameliorates GVHD in murine models (19).

Alternatively it could be argued that the elevation of IL-18BP as well as of IL-18, sIL-6R, and sIFN-γR levels observed in association with acute GVHD is just an epiphenomenon and not a cause–effect mechanism. Whatever the interpretation, it is evident that IL-18BP levels may be used as a diagnostic tool for assessing engraftment and transplant-related complications.

In conclusion, our findings indicate that: (1) IL-18BP levels decline during conditioning and then increase in direct correlation with engraftment, (2) IL-18BP and IL-18 serum levels are markedly elevated in association with acute GVHD, and (3) IL-18BP levels correlate both with sIL-6R and sIFN-γR levels. Taken together our data suggest that dysregulation of IL-18 and IL-18BP may play an important role in the pathophysiology of transplant-related complications and therefore represent potential targets for the development of new therapeutic strategies in acute GVHD.

REFERENCES


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Received June 28, 2001; accepted August 22, 2001.