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Identification of gene microarray expression profiles in patients with chronic graft-versus-host disease following allogeneic hematopoietic cell transplantation

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Abstract Chronic graft-versus-host disease (GVHD) results in significant morbidity and mortality, limiting the benefit of allogeneic hematopoietic cell transplantation (HCT). Peripheral blood gene expression profiling of the donor immune repertoire following HCT may provide associated genes and pathways thereby improving the pathophysiologic understanding of chronic GVHD. We profiled 70 patients and identified candidate genes that provided mechanistic insight in the biologic pathways that underlie chronic GVHD. Our data revealed that the dominant gene signature in patients with chronic GVHD represented compensatory responses that control inflammation and included the interleukin-1 decoy receptor, IL-1 receptor type II, and genes that were profibrotic and associated with the IL-4, IL-6 and IL-10 signaling pathways. In addition, we identified three genes that were important regulators of extracellular matrix. Validation of this discovery phase study will determine if the identified genes have diagnostic, prognostic or therapeutic implications.

Abbreviations: GVHD, graft-versus-host disease; HCT, allogeneic hematopoietic cell transplantation; IL-1, interleukin-1; IL-1R2, IL-1 receptor type II; RIC, reduced intensity conditioning; PBMC, peripheral blood mononuclear cells; cDNA, complimentary DNA; cRNA, complimentary RNA; PAM, Prediction Analysis of Microarray; KEGG, Kyoto Encyclopedia of Genes and Genomes; SLE, systemic lupus erythematosus; OLP, oral lichen planus

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1. Introduction

Chronic graft-versus-host disease (GVHD) is the major late complication after allogeneic hematopoietic cell transplantation (HCT) and approximately half of patients who survive beyond 100 days after transplantation develop chronic GVHD [1,2]. The manifestations of chronic GVHD adversely impact patient quality of life and the three year survival rate for newly diagnosed "favorable" and "poor" risk chronic GVHD is approximately 80% and 40%, respectively [3] and only 50% of patients discontinue immunosuppressive treatment within 5 years after its onset [4,5]. Improvements in supportive care and infection prophylaxis and the introduction of reduced intensity conditioning (RIC) have led to a significantly higher number of patients surviving beyond 100 days after transplant [6–10], and therefore the number of patients at risk for chronic GVHD was estimated to double over a 5 year period [11].

The pathogenesis of chronic GVHD is incompletely understood and although it often follows acute GVHD, it is clinically and biologically distinct, and can occur even in the absence of acute GVHD [12]. Chronic GVHD is predominantly a disease of immune dysregulation with donor T cells mediating chronic alloimmune (directed to recipient tissue histocompatibility antigens) and autoimmune (directed against antigens on both donor and recipient tissue) reactions [13,14]. The activated donor immune response progresses because of attenuated or absent thymic and peripheral mechanisms of clonal deletion and tolerance [15,16]. The pathologic immune response attacks the target tissues of chronic GVHD through direct cellular mechanisms, and inflammatory and sclerosing cytokines, and autoantibody production [17,18].

The diagnosis of chronic GVHD can be somewhat challenging especially when diagnostic features are absent or when the clinical features are confined to internal organs (i.e., lungs) or clinical assessment is hampered by medical co-morbidities. In the more challenging cases biopsy confirmation is essential to the diagnosis [19]. About 10% of patients referred to a chronic GVHD specialist had no biopsy and were incorrectly diagnosed and treated prior to their referral [20]. Furthermore, uniform minimal diagnostic histologic criteria for chronic GVHD have not been established and validated for affected organs, and sampling and technical factors can also contribute to a false negative histologic assessment. Due to the diagnostic challenges in the clinical presentation and the limitations in time and invasiveness of obtaining a tissue biopsy, a recent focus has been to identify surrogate diagnostic markers of chronic GVHD [21–23]. To date, no single gene or pathway has been identified, validated, and introduced into clinical practice for chronic GVHD.

Discovery phase gene expression microarray profiles provide a snapshot of all the transcriptional activity in a biological condition, and facilitate the identification of novel and previously unrecognized functional role of genes. In the current report, we conducted a discovery phase gene expression study and hypothesized that the gene expression profile of the circulating donor immune cell repertoire following allogeneic HCT would highlight a list of candidate genes and pathways associated with the presence of chronic GVHD. Among 70 patient samples assessed in this retrospective cross-sectional, discovery phase study, we identified potential genes that appeared consistent with the pathobiology and cellular basis of chronic GVHD. We used biostatistical methods and repeated the analyses 100 times with random different splits of the confounding gene elimination, training and tests sets, and consistently identified a repertoire of genes that were regulators of excessive inflammatory bioactivity included the interleukin-1 (IL-1) decoy receptor, IL-1 receptor type II (IL-1R2), and genes that were profibrotic and associated with the IL-4, IL-6 and IL-10 signaling pathways. In addition, we identified three genes that were important regulators of extracellular matrix remodeling involved in connective tissue disorders. Our data suggests that the dominant gene signature in patients with established chronic GVHD represent compensatory anti-inflammatory and profibrotic genes. This discovery phase study supports the utility of gene profiling to further the understanding of the molecular pathogenesis of chronic GVHD and following further refinements may represent a useful noninvasive assay in the diagnosis, prognostic assessment, and identification of novel therapeutic approaches in chronic GVHD.

2. Materials and methods

2.1. Study population

The Division of Blood and Marrow Transplantation at Stanford University Medical Center maintains a comprehensive clinical and pathologic database of patients undergoing transplantation on Institutional Review Board approved protocols. Peripheral blood samples for research purposes are routinely obtained from patients at designated time points after transplant following written informed consent. A computer review of the database identified 167 patients between February 13, 2008 and April 24, 2009, who were seen in the outpatient clinic and met the following inclusion criteria: alive beyond 100 days from allogeneic HCT with complete (100%) donor $CD3^+$ T cell chimerism, without disease relapse for greater than 90 days after the time of sample acquisition, and for which research blood samples were available. The exclusion criteria included patients with active viral, fungal, or bacterial infection or who received treatment for documented viral (including viremia), fungal, or bacterial infection within 30 days of sample acquisition. Patients classified in the non-chronic GVHD group had an additional exclusion which was the development of GVHD within 90 days of sample acquisition. From among this group, 68 of 167 patients were conveniently selected for analysis in the current study.

The information in the database is gathered from the chart note following a medical assessment by the primary transplant physician during the clinic visit. A review of the medical records of the 68 patients was performed by two independent investigators to confirm patient inclusion and exclusion criteria, confirm a history of acute and chronic GVHD and its severity, and the presence or absence of chronic GVHD at the time of sample acquisition. The reviewed information also included the immune suppression medication(s), if any, prescribed for GVHD therapy and its dose(s), the peripheral blood leukocyte count and differential, and the status of chimerism and disease. From among the cohort of 68 patients, 3 were excluded due to disease relapse within 90 days of the sample acquisition date, and 2 were excluded because of