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Understanding Gene Expression Patterns in Immune-Mediated Disorders

Russell L. Dedrick
X Dx, Inc., San Francisco, California

Gene expression profiling of peripheral blood cells can provide dynamic information regarding the host response to immune-mediated disorders. AlloMap® molecular expression testing from XDx monitors the expression of 20 genes in peripheral blood mononuclear cells (PBMC) to discriminate cardiac allograft recipients of 15 years or greater who are at low risk for acute cellular rejection (ACR). The AlloMap test classifier is based on the expression level of 11 genes, encoding proteins with diverse functions, which are differentially expressed in stable patients with moderate to severe ACR compared to patients without ACR. The nine other test genes are used for normalizing gene expression levels and assuring sample quality. In this work we review the development processes leading to the selection of the 11 informative genes and the derivation of the AlloMap test classifier, and discuss the relationship of peripheral blood gene expression with diverse pathways associated with ACR, including T-cell priming, platelet activation, systemic responses to allograft inflammation, and the overall state of immunosuppression.

Keywords AlloMap, gene expression profiling, cardiac allograft rejection

INTRODUCTION

Cardiac allograft transplantation is an established treatment in many cases of end-stage heart disease. However, careful vigilance must be maintained after transplantation to achieve sufficient immunosuppression to prevent acute cellular rejection (ACR) while minimizing side effects associated with the medication. The current management of heart transplant patients includes frequent surveillance and symptom-driven biopsy of the endomyocardium (Billingham et al., 1990; Stewart et al., 2005). Endomyocardial biopsy is an expensive and invasive technique subject to sampling error, with documented variability in the pathologic interpretation of the biopsy specimens (Baraldi-Junkins et al., 1993; Winters and McManus, 1996). To minimize the need for biopsy in stable cardiac allograft recipients who are highly unlikely to be experiencing moderate-to-severe ACR, differential patterns of gene expression in peripheral blood mononuclear cells (PBMC) were utilized to develop the non-invasive AlloMap molecular expression testing (Deng et al., 2006).

Immunoregulatory Pathways in Transplantation

ACR occurs when recipient T-lymphocytes are stimulated by the genetically dissimilar proteins of the donor allograft (Rose, 1998). The rejection response is thought to be initiated by pre-existing memory T-lymphocytes that cross react with the alloantigens (Chalasani et al., 2002; Shiao et al., 2005), but is further amplified by activation of higher affinity naïve T-lymphocytes. The activated T-lymphocytes infiltrate the transplanted organ and initiate an inflammatory response, producing mediators that induce expression of adhesion molecules and chemokines on the graft endothelium. The activated endothelium recruits additional immune cells into the allograft, stimulating donor and recipient dendritic cells to mature and carry allograft antigens to the lymph node.

Normally, naïve T-lymphocytes can only leave the circulation at the specialized vascular endothelium of the lymph node. When stimulated by the antigen-loaded mature dendritic cell in the lymph node tissue, allo-reactive naïve T-lymphocytes proliferate and acquire new trafficking and effector functions. These primed effector T-lymphocytes re-enter the circulation until they encounter the inflamed allograft where they can infiltrate and further amplify the graft-reactive immune response (Saiki et al., 2001). Immunosuppressive drugs in current usage target various stages of this process by inhibiting T-lymphocyte activation (Kiani et al., 2000), proliferation (Quemener et al., 2004), and the inflammatory processes that lead to activation of endothelial and dendritic cells (Ray and Searle, 1997; Piemonti et al., 1999).
The conversion of a T-lymphocyte from a naïve to an activated state is a critical process in the adaptive immune response, and requires two distinct signaling events involving molecules on the surface of the T-lymphocytes and cognate receptors on dendritic cells, which function as antigen-presenting cell (APC). Activation is initiated when the T-cell receptor (TCR) recognizes the complex of an antigenic peptide bound to a major histocompatibility complex (MHC) protein on the APC. The second signaling event is mediated by one of several co-stimulatory interactions between the T-lymphocyte and APC. The best-characterized co-stimulatory pathways involve proteins of the CD28 family expressed on the T-lymphocytes and their receptors expressed on the APC. Co-stimulatory receptors expressed on T-lymphocytes can transmit either positive signals via CD28 or inducible co-stimulator (ICOS) molecules, or negative signals via cytotoxic lymphocyte antigen-4 (CTLA4), B- and T-lymphocyte attenuator (BTLA) or programmed death-1 (PD-1) (Sharpe and Freeman, 2002). The net co-stimulatory signal provides an important balance between an effective immune response and limiting auto-reactivity.

**AlloMap Molecular Expression Testing**

Differential gene expression in PBMC in response to ACR was used to develop the AlloMap test classifier in cardiac allograft recipients. The Cardiac Allograft Rejection Gene Expression Observational (CARGO) Study enrolled 629 patients and collected 4,917 blood samples in association with endomyocardial biopsy specimens and clinical data (Deng et al., 2006). The biopsy grade was established using the 1990 grading system approved by the International Society of Heart and Lung Transplantation (ISHLT) (Billingham et al., 1990) after evaluation by the local pathologist and three independent pathologists who were blinded to the clinical data. PBMC RNA was prepared from two patient groups, those whose biopsy showed no ACR (grade = 0) and those with moderate-to-severe ACR (grade ≥ 3A). Candidate genes were selected by differential expression between the two patient categories using custom microarrays and were supplement with genes that had been associated with the process of rejection in the literature.

Quantitative real-time PCR (qRT-PCR) assays were developed for 252 genes, and 68 genes were identified that showed differential expression between the no ACR and the moderate to severe ACR patient groups. Differential gene expression was defined as statistically significantly increased or decreased expression in one group compared to the other. From these genes, a classifier was derived using linear discriminant analysis that combined the expression level of 11 genes (Table 1) to provide the best discrimination between the two clinical groups ($p = 0.0018$). The classifier contains seven terms, including four individual genes and the three metagenes where the expression level of coordinately expressed genes is averaged (Table 2). The AlloMap classifier combines the gene expression information into a single score ranging from 0 to 40, with lower scores corresponding to lower risk of ACR. The expression levels of nine additional genes that are not affected by ACR.

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### TABLE 1
Genes of the AlloMap Test

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>R/NR</th>
<th>$t$-test $p$-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDCD1</td>
<td>1.46</td>
<td>$1.6 \times 10^{-5}$</td>
<td>Programmed cell death-1</td>
</tr>
<tr>
<td>SEMA7A</td>
<td>1.29</td>
<td>$6.3 \times 10^{-5}$</td>
<td>Semaphorin 7A</td>
</tr>
<tr>
<td>ITGA4</td>
<td>1.18</td>
<td>0.0011</td>
<td>$\alpha_4$ integrin, the alpha subunit of VLA-4</td>
</tr>
<tr>
<td>RHOU</td>
<td>1.20</td>
<td>0.0008</td>
<td>GTPase of the RHO family (AHRU)</td>
</tr>
<tr>
<td>MARCH8</td>
<td>1.14</td>
<td>0.03</td>
<td>E3 ubiquitin ligase (E-MIR, MIR)</td>
</tr>
<tr>
<td>WDR40A</td>
<td>1.16</td>
<td>0.11</td>
<td>WD repeat domain 40A</td>
</tr>
<tr>
<td>ITGAM</td>
<td>0.85</td>
<td>$3.6 \times 10^{-4}$</td>
<td>$\alpha_M$ integrin, CD11b, alpha subunit of MAC-1</td>
</tr>
<tr>
<td>FLT3</td>
<td>0.60</td>
<td>0.0020</td>
<td>fms-related tyrosine kinase 3</td>
</tr>
<tr>
<td>IL1R2</td>
<td>0.48</td>
<td>$6.4 \times 10^{-5}$</td>
<td>Interleukin 1 type II decoy receptor</td>
</tr>
<tr>
<td>PF4</td>
<td>0.74</td>
<td>0.0032</td>
<td>Platelet factor 4</td>
</tr>
<tr>
<td>C6orf25</td>
<td>0.67</td>
<td>0.0068</td>
<td>Immunoglobulin superfamily member located in the MHC class III region (G6b)</td>
</tr>
</tbody>
</table>

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1. Official symbol as designated by the National Center for Biotechnology Information.
2. Ratio of median expression level in R, patients with moderate-to-severe ACR, and NR, patients with no evident ACR (Deng et al., 2006).

### TABLE 2
Terms of the AlloMap test

<table>
<thead>
<tr>
<th>Terms</th>
<th>Expression in Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Gene Terms</td>
<td></td>
</tr>
<tr>
<td>PDCD1</td>
<td>Increased</td>
</tr>
<tr>
<td>SEMA7A</td>
<td>Increased</td>
</tr>
<tr>
<td>ITGA4</td>
<td>Increased</td>
</tr>
<tr>
<td>RHOU</td>
<td>Increased</td>
</tr>
<tr>
<td>Metagene Terms</td>
<td></td>
</tr>
<tr>
<td>PF4, C6orf25</td>
<td>Decreased</td>
</tr>
<tr>
<td>MARCH8, WDR40A</td>
<td>Increased</td>
</tr>
<tr>
<td>ITGAM, FLT3, IL1R2</td>
<td>Decreased</td>
</tr>
</tbody>
</table>
are used to quality control each test and to allow normalize expression levels between samples.

In practice, a patient has a blood sample collected by routine phlebotomy and PBMC are prepared by density gradient centrifugation and lysed in extraction buffer by the local laboratory. This lysate is shipped overnight to the reference laboratory where the RNA is purified and mRNA levels for the test genes are measured by qRT-PCR. Expression levels are normalized using the test control genes, and the AlloMap algorithm is used to calculate a score that is reported back to the requesting physician. This information is considered along with other clinical parameters to assess the patient's risk for ACR.

**Genes of the AlloMap Test**

**PDCD1.** Initially identified in a T-lymphocyte hybridoma undergoing apoptosis, PDCD1 (programmed cell death 1) encodes a cell surface protein, PD-1, expressed on T-lymphocytes, B-lymphocytes and myeloid cells after activation (Agata et al., 1996). PD-1 is a member of the CD28 family of T-lymphocyte co-stimulatory proteins and its function is best characterized in the context of T-lymphocyte activation. In contrast to the costimulatory activity of CD28, PD-1 inhibits T-cell receptor-mediated activation, including both proliferation and cytokine production, when engaged by one of its ligands, PD-L1 or PD-L2 (Freeman et al., 2000; Latchman et al., 2001). PD-1 crosslinking also inhibits proliferation of B-lymphocytes stimulated through the antigen receptor (Zhong et al., 2004). Reduced proliferation is associated with cell cycle arrest, and not an increase in apoptosis (Latchman et al., 2001), as the gene name might suggest.

The PD-1 ligands are expressed on activated T-lymphocytes, monocytes and dendritic cells, microvascular endothelium, thymic epithelium and placental syncytiotrophoblasts, indicating roles for the PD-1 pathway in T-lymphocyte development, antigen presentation, peripheral tolerance and maternal fetal tolerance. PD-1 deficient mice are susceptible to autoimmune disorders, such as arthritis and glomerulonephritis (Nishimura et al., 1999), and cardiomyopathy (Nishimura et al., 2001), suggesting that the PD-1 pathway functions to limit reactivity to self antigens. In murine allograft models, expression of PD-1 and its ligands is induced in cardiac allografts, but not isografts (Ozkaynak et al., 2002). Stimulating this pathway delayed rejection in conjunction with a subtherapeutic dose of cyclosporine A demonstrating a role of the PD-1 signaling pathway in regulating intragraft immune activation.

**ITGA4.** Immune reactions in peripheral tissues depend on molecular mechanisms that direct the trafficking of leukocytes to particular sites. Selectins, chemokines and integrins direct cells to leave the blood flow and adhere to, and subsequently infiltrate through the endothelial lining of the blood vessel into the underlying tissue. The ITGA4 gene encodes the α4 integrin (CD49d), which can combine with either the β1 integrin subunit (CD29) to form VLA-4 (very late antigen 4), or with the β1 integrin subunit to form the α4β7 integrin (Springer, 1994). Both of these integrin molecules are primarily expressed by leukocytes.

The VLA-4 integrin is of particular interest in cardiac allograft transplantation because its ligands, VCAM-1 (vascular cell adhesion molecule 1) and an alternately spliced form of fibronectin (connecting segment (CS)-1) are increased at sites of inflammation (Osborn et al., 1989; Elsees et al., 1990; Coito et al., 1997). Naïve T-lymphocytes are only able to leave the circulation to enter lymph nodes, where they are exposed to antigens presented by dendritic cells. VLA-4 expression is increased on T-lymphocytes by activation, reflecting the reprogrammed trafficking properties that allow memory and effector T-lymphocytes to infiltrate inflamed tissues, such as a rejecting allograft. Blocking VLA-4 function prolonged cardiac allograft survival in a murine model confirming that the VLA-4 pathway plays a significant role in the rejection process (Isobe et al., 1994).

The α4β7 integrin participates in the routine trafficking of lymphocytes to mucosal tissues and Peyer's patches.

**SEMA7A.** SEMA7A encodes a glycosylphosphatidylinositol tol-linked protein, semaphorin 7A (Sema7A), that is a member of the semaphorin protein family (Xu et al., 1998). Semaphorins were first identified for their role in controlling neuronal axon outgrowth, but have subsequently been shown to function in the immune system. SEMA7A is expressed both on neuronal tissues, lymphoid cells, myeloid cells and erythrocytes (Bobolis et al., 1992; Xu et al., 1998). Expression is enhanced on CD4 T cells and dendritic cells by activation. Hyper-responsiveness of T-lymphocytes in mice deficient for Sema7A suggests this protein functions to negatively regulate T-lymphocyte activation (Czopik et al., 2006).

**RHO1 [ARHU].** The RHOU gene encodes the Wrch-1 (Wnt-regulated Cdc42 homolog) protein, which is a novel member of the Rho GTPase subfamily. GTPases are molecular switch proteins that cycle between an active state bound to GTP and an inactive state produced when the inherent GTPase hydrolyzes the GTP to GDP (Etienne-Manneville and Hall, 2002). In the active state, the GTPase proteins bind other target proteins and stimulate them to generate a response until GTP hydrolysis inactivates the GTPase component. The active GTPase is then regenerated when a stimulus induces a member of the guanine nucleotide exchange factor (GEF) family to replace the GDP with a fresh GTP molecule. Mammalian cells contain several hundred different GTPase molecules that are involved in a wide variety of functions.

The Rho GTPase subfamily regulates controlled assembly of cytoskeletal elements and participates in cellular activities such as the establishment of cell morphology, vesicular transport, neurite outgrowth, cell migration, and phagocytosis. Rho GTPases also participate in signal transduction pathways, such as T-lymphocyte activation, that lead to proliferation. The Wrch-1 protein is expressed in a variety of tissues, including brain, skeletal muscle and placenta (Tao et al., 2001). Over-expression of Wrch-1 in cultured epithelial cells stimulates quiescent cells to enter the cell cycle and alters cytoskeletal organization. The function of RHOU in the immune system is not well understood.
MARCH8 [MIR, c-MIR]. The MARCH8 gene encodes a protein of the E3 ubiquitin ligase family (Goto et al., 2003). This family of molecules regulates protein turnover by targeting other proteins for ubiquitination that leads to intracellular degradation. MARCH8 is the cellular homolog of two genes from the Karpov's sarcoma-associated-retrovirus called modulator of immune recognition (MIR) 1 and 2. The viral MIR1 and 2 ligases target proteins that regulate the immune response such as major histocompatibility complex class I (MHC-I), the costimulatory ligand B7.2 and the intracellular adhesion molecule 1 (ICAM-1). Ubiquitination of these proteins stimulates their removal from the cell surface and helps the virus evade the host immune system. A similar class of target proteins has been identified for the MARCH8 gene product. In transfected HeLa cells, substrates for MARCH8 include the MHC-I protein HLA-A2.1, Fas (CD95), the transferrin receptor, and B7.2 (Bartee et al., 2004). The MARCH8 gene is expressed in a variety of tissues, including lung, kidney, pancreas and placenta, and although its precise physiological role is not well understood, it is thought to be involved in modulating immune reactivity.

WDR40A. The WDR40A gene encodes a member of the WD-repeat protein family. These proteins contain conserved repeating units of 23-41 amino acids that typically end with the dipeptide tryptophan-aspartic acid (WD) (Neeh et al., 1994). Structurally, the WD-repeat segments have been shown to fold into a multi-bladed β-propeller domain (Sondek et al., 1996). While proteins of the WD-repeat family tend to have regulatory functions, a specific function of the WDR40A protein has not been identified.

IL1R2. The IL1R2 gene encodes a non-signaling, decoy receptor for the potent inflammatory cytokine, interleukin 1 (IL-1) (Colotta et al., 1993). Two forms of IL-1, IL-1α and IL-1β, are expressed from two different genes, but the proteins have similar properties that promote inflammation by inducing expression of adhesion molecules, cytokines, chemokines, and other inflammatory mediators on target cells (Dinarello, 1996). IL-1 expression is induced by a number of inflammatory stimuli, including infection, ischemia-reperfusion and acute cellular rejection. Intracellular signaling is initiated when either form of IL-1 binds to the type I IL-1 receptor (IL-1R1) and the complex recruits a third molecule, the IL-1 receptor accessory protein (IL-1AcP). The type II IL-1 receptor, the product of the IL1R2 gene, can bind either form of IL-1, but does initiate signaling. This effectively sequesters IL-1 from the signaling receptors and modulates the inflammatory activity of the cytokine (Mantovani et al., 2001).

The decay molecule is the predominant IL-1 receptor expressed on the surface of neutrophils, monocytes and B-lymphocytes. The extracellular domains of the receptor can also be shed as a soluble antagonist of IL-1 (Wang et al., 1998). Expression of IL1R2 is transcriptionally induced by glucocorticoids such as dexamethasone (Re et al., 1994).

ITGAM. The ITGAM gene encodes αM integrin (CD11b), providing a second member of the integrin gene family (along with ITGA4) to the discriminator genes of the AlloMap test. The αM subunit forms a heterodimer with the β2 integrin protein (CD18) to form the adhesion molecule known as Mac-1, complement receptor type 3 (CR3). Mac-1 is expressed primarily on myeloid cells such as monocytes, neutrophils and dendritic cells, along with NK cells and a small subset of B-lymphocytes and T-lymphocytes (Muto et al., 1993; Springer, 1994). Ligands for the Mac-1 complex include intracellular adhesion molecule-1 (ICAM-1), fibrinogen, and microorganisms opsonized with the factor I-cleaved C3b complement fragment of C3 (iC3b)(Vetvicka et al., 1996). Thus, the Mac-1 integrin functions in both cell trafficking and phagocytic processes. In addition to basal cell surface expression, monocytes and neutrophils contain intracellular stores of Mac-1 in vesicular compartments that are rapidly mobilized to the plasma membrane in response to activation (Miller et al., 1987). In cardiac transplant patients, elevated expression of CD11b on neutrophils measured in preoperative blood samples correlated with the rejection grade at the first post-transplant biopsy (Healy et al., 2006).

FLT3. The FLT3 gene encodes the fms-related tyrosine kinase receptor (FLT3). FLT3 is expressed primarily on early hematopoietic progenitors in the bone marrow (Small et al., 1994; Turner et al., 1996). FLT3 serves as a receptor for FLT3 ligand, a factor produced by bone marrow stromal cells that synergizes with other growth factors and cytokines to stimulate proliferation of stem cells, progenitor cells, dendritic cells, and natural killer cells (McKenna et al., 2000). Administration of FLT3 ligand increases dendritic cell numbers in both lymphoid and non-lymphoid organs (Maraskovsky et al., 1996). In mouse models of cardiac transplantation, administration of Flt3 ligand to donor mice prior to transplantation accelerated the rejection response (Steptoe et al., 1997).

PF4. The PF4 gene encodes platelet factor 4, a member of the CXC chemokine family. Platelet factor 4 is a major component of the platelet α-storage granule, and large amounts are released into the surrounding medium when platelets are activated (Flieder et al., 1981). Expression in other cell types, including monocytes (Schaffer et al., 2005) has also been described. Unlike the other members of the CXC chemokine family, PF4 has poor chemotactic activity for any known cell and its function is not well understood. Reported activities include inhibition of endothelial cell proliferation, neutrophil activation, modulation of T-lymphocyte activity and altering the structure of fibrin during clot formation (Maione et al., 1990; Petersen et al., 1998; Fleischer et al., 2002; Liu et al., 2005). Although many of the properties of PF4 have been attributed to its high affinity for heparin, heparan sulfate and other negatively charged proteoglycans (Levine and Wohl, 1976; Petersen et al., 1998), a splice variant of the CXCRR3 receptor has recently been identified, CXCR3B, that binds PF4 and may mediate some of its biological activities (Lasagna et al., 2003).

C6orf25 [G6h]. The C6orf25 gene is located in the class III region of the major histocompatibility complex and encodes a member of the immunoglobulin superfamily (Ribas et al., 1999; de Vet et al., 2001). Structural analysis indicates that the
C6orf25 gene encodes a cell surface protein, G6b, with a single extracellular immunoglobulin V-like domain followed by a hydrophobic transmembrane segment and two immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the cytoplasmic tail. Two splice variants lacking the putative transmembrane segment were shown to be secreted, and the extracelluar domain has been shown to have a high affinity for heparin, a highly sulfated, negatively charged glycosaminoglycan widely expressed in the extracellular matrix and on cell surfaces (de Vet et al., 2005). Documented expression of the C6orf25 gene is limited to one erythroleukemia (K562) and two T-cell leukemia (Molt4 and Jurkat) cell lines. Although the structure suggests that G6b may be a receptor, its physiologic function is not known.

Understanding the Relationship of Gene Expression Patterns and the Process of Acute Cellular Rejection

The CARGO study demonstrated that moderate-to-severe ACR in stable cardiac allograft recipients results in differential patterns of gene expression in PBMC compared to stable patients without ACR. Gene expression patterns in peripheral blood cells may be influenced by several factors, including the process of rejection, effects of immunosuppressive drugs, and/or a systemic response to the rejecting organ. Although 68 genes differentially expressed genes were identified, linear discriminant analysis (LDA) generated an optimized classifier that best distinguished rejection from non-rejection based on the expression levels of 11 genes as measured by qRT-PCR. The AlloMap test classifier is composed of 7 terms representing diverse molecular pathways, including 4 terms based on the expression level of individual genes, and 3 metagenes comprised of the average expression level of coordinately expressed genes is used (see Table 2).

The immunobiology of the ACR responsive AlloMap test genes provides clues as to how the observed changes in gene expression reflect the underlying process of acute cellular rejection. ACR is mediated by alloreactive recipient T-lymphocytes that are stimulated by the genetically dissimilar transplanted organ. The PD1 gene is known to be induced on activated T-lymphocytes and recent evidence suggests that the protein encoded by this gene is present on circulating, antigen-specific T-lymphocytes only during the course of an active immune response (Barber et al., 2006). The elevated level of PD1 expression is observed in PBMC from rejection samples may indicate an increasing number of circulating T-lymphocytes that have recently undergone activation in the lymph node. Elevated expression of PD1 in patients experiencing ACR may reflect inadequate immunosuppression and breakthrough activation of graft-reactive T-lymphocytes. Similarly, increased expression of ITGA4, an integrin molecule that permits T-lymphocytes to traffic to sites of inflammation, is also consistent with elevated T-lymphocyte immune activity. Expression of other genes that are typically associated with effector T-lymphocytes, perforin, granzyme and FasL, was also significantly elevated in the CARGO study, but these genes were not included in the final classifier because either they were less discriminating or the mRNAs were less stable during sample processing (Deng et al., 2006).

In the AlloMap test classifier use the average expression level of genes that are highly coordinately expressed and therefore are likely to be providing similar mechanistic information. One of the metagene terms is composed of the genes IL1R2, FLT3 and ITGAM. The IL1R2 gene is transcriptionally induced by glucocorticoids (Re et al., 1994). Analysis of samples from the CARGO study demonstrated that expression levels of IL1R2, FLT3 and ITGAM all correlated with steroid dosage (data not shown). These genes are expressed at lower levels in moderate to severe ACR, even when steroid doses are similar, and may reflect an inadequate response to corticosteroids.

The coordinately expressed genes PF4 and C6orf25 (G6b) comprise a second metagene term. Preliminary cell fractionation experiments using PBMC samples from healthy donors indicate that platelets are primary source of these two genes. It has been observed previously that more severe grades of ACR are associated with higher levels of platelet activation (Segal et al., 2001) in blood samples collected from cardiac allograft recipients. Differential expression of these genes, which is reduced in moderate to severe ACR, may reflect this phenomenon. Although several blood cells express the genes of the final metagene term, MARCH8 and WDR40A, the primary source in a PBMC sample seems to be residual reticulocytes. ACR is associated with higher levels of these genes, and this may reflect inflammation-induced erythropoiesis (Petakov et al., 1998) in response to the rejection response.

The ability to examine the transcriptional activity of the entire human genome in blood cells provides exciting opportunities to identify useful markers for less invasive diagnostic tests and to learn more about the systemic effects of immune-mediated disorders. PBMC are composed of several different cell types, including, T, B-, and NK lymphocytes, monocytes, platelets, and immature forms of granulocytes and reticulocytes. Levels of mRNA in this tissue source can be affected both by the pattern of genes expressed by these different cell types, and by the relative numbers of each cell type in the PBMC sample. Both the function and the source of genes provide clues for understanding the biological pathways underlying differential expression patterns in different clinical indications. Thus, expression levels of the genes monitored by the AlloMap test seem to be influenced by several distinct pathways associated with ACR, including T-lymphocyte priming, platelet activation, inflammation and the functional effects of immunosuppression. A better understanding of these molecular signals could lead to diagnostic tools in new clinical indications and help physicians optimize treatment for their individual patients.

Note added in proof: Expression of the G6b protein was recently documented in platelets (Senis et al., 2007).

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