this novel approach to allogeneic transplantation. We believe that it is important to promote an evidence-based evolutionary approach, rather than a creationist approach, to transplantation research.

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To the editor:

Gene expression profiling of the functionally distinct human bone marrow stromal cell lines HS-5 and HS-27a

Two human stromal cell lines, HS-5 and HS-27a, represent functionally distinct components of the bone marrow microenvironment.[1,2] HS-27a supports cobblestone area formation by early hematopoietic progenitors, whereas HS-5 secretes multiple cytokines that support the proliferation of committed progenitors. These cell lines, which are available from the American Type Culture Collection (ATCC, Manassas, VA), have been distributed to research groups worldwide for use as a tool to understand interactions between hematopoietic cells and their microenvironment. We have recently used DNA microarray technology to characterize and compare the expression of over 17 000 genes in these cell lines.

Briefly, microarray construction and hybridization protocols were modified from Marton et al.[3] The microarrays were constructed using a set of more than 17 000 sequence-verified clones from Research Genetics (Huntsville, AL). Of the 17 761 features (spots) on the microarray, 186 are control nonexpressed or nonhuman sequences or housekeeping genes. UniGene cluster IDs could

be assigned by GenBank to 16 592 of the features as of June 1, 2001 (UniGene Build 133), indicating that they are representatives of nonredundant unique genes. Many have been functionally characterized, and chromosomal location and tissue expression patterns are known for others. Total RNA was isolated from semiconfluent cultures and reverse-transcribed into cDNA in a nucleotide mix containing amino-allyl deoxyuracil triphosphate (dUTP). The cDNA from stromal cells and Universal RNA (Stratagene, La Jolla, CA) was covalently coupled separately with Cy5 and Cy3 monoreactive fluors, respectively. The Universal RNA consists of a mixture of RNA from 10 different human cell lines with a broad expression coverage of over 80% of the sequences on the array, allowing comparison of expression patterns of multiple different samples of different origin. The Cy5- and Cy3-labeled cDNAs were combined for hybridization to the microarray. Fluorescent array images were collected for both Cy3

Table 1. Transcripts with greater than 10-fold higher expression in HS-27a than in HS-5

Clone ID	Accession	Cluster ID	Name	Annotation	HS-27a/HS-5*
80643	T57803	Hs.10283	RBM8B	RNA binding motif protein 8B	83.6
22355	T89094	Hs.227571	RGS4	Regulator of G-protein signalling 4	77.7
753982	AA479967	Hs.7882	NA	EST	30.7
429349	AA007419	Hs.227571	RGS4	Regulator of G-protein signalling 4	28.2
366801	AA029430	Hs.61557	NA	EST	27.8
784337	AA447115	Hs.237356	SDF1	Stromal cell-derived factor 1	25.6
139354	R63735	Hs.15093	HSPC195	Hypothetical protein	24.4
841238	AA487121	Hs.237868	IL7R	Interleukin 7 receptor	24.1
68605	T53298	Hs.119206	IGFBP7	Insulin-like growth factor binding protein 7	23.5
897910	AA598653	Hs.136348	OSF-2	Osteoblast specific factor 2 (fasciclin I-like)	21.0
489631	AA101875	Hs.81800	CSPG2	Chondroitin sulfate proteoglycan 2 (versican)	19.6
840460	AA485865	Hs.237868	IL7R	Interleukin 7 receptor	19.3
898218	AA598601	Hs.77326	IGFBP3	Insulin-like growth factor binding protein 3	18.6
755612	AA419229	Hs.85339	GPR39	G protein-coupled receptor 39	17.2
773392	AA425749	Hs.2799	CRTL1	Cartilage linking protein 1	16.7
489519	AA099153	Hs.245188	TIMP3	Tissue inhibitor of metalloproteinase 3	16.4
302766	N90744	Hs.293907	FLJ23403	Hypothetical protein FLJ23403	15.1
743146	AA401309	Hs.293907	FLJ23403	Hypothetical protein FLJ23403	13.6
809719	AA455497	Hs.170121	NA	EST	13.4
133114	R26355	Hs.19545	FZD4	Frizzled (Drosophila) homolog 4	11.7
1492230	AA875933	Hs.76224	EFEMP1	EGF-containing fibulinlike ECM protein 1	11.6
796170	AA461086	Hs.16578	NA	EST	11.2
140197	R66101	Hs.103291	LOC51299	Neuritin	10.3
731311	AA416767	Hs.170121	NA	EST	10.3
309583	N94424	Hs.82547	RARRES1	Retinoic acid receptor responder	10.2

Identifiers, names, and functional descriptions are derived from information available in public databases, primarily the National Center for Biotechnology Information (NCBI) UniGene database and GenBank. NA indicates name not available.

EST represents expressed sequence tag; EGF, epidermal growth factor; and ECM, extracellular matrix.

*Average value in gene expression of HS-27a (n = 4) was divided by average value in gene expression of HS-5 (n = 4). Sequences that appear twice were specified by 2 different clones on the microarray.

Table 2. Transcripts with greater than 10-fold lower expression in HS-27a than in HS-5

Clone ID	Accession	Cluster ID	Name	Annotation	HS-27a/HS5*
845477	AA644211	Hs.196384	PTGS2	Prostaglandin-endoperoxide synthase 2	0.003
147050	R80217	Hs.196384	PTGS2	Prostaglandin-endoperoxide synthase 2	0.004
310406	N98591	Hs.93913	IL6	Interleukin 6	0.006
768417	AA495835	Hs.103839	EPB41L3	Erythrocyte membrane protein band 4.1-like 3	0.010
324655	W47101	Hs.126256	IL1B	Interleukin 1, beta	0.012
767167	AA424568	Hs.42500	ARL5	ADP-ribosylation factor-like 5	0.017
503583	AA131240	Hs.297880	NA	EST	0.019
726086	AA399473	Hs.295944	TFPI2	Tissue factor pathway inhibitor 2	0.019
70692	T49159	Hs.75716	SERPINB2	Serine proteinase inhibitor, clade B, member 2	0.021
491763	AA150507	Hs.126256	IL1B	Interleukin 1, beta	0.021
323238	W42723	Hs.789	GRO1	GRO1 oncogene	0.023
773330	AA425450	Hs.82226	GPNMB	Glycoprotein (transmembrane) nmb	0.027
261204	H98218	Hs.308780	NA	EST	0.030
246786	N53172	Hs.23016	RDC1	G protein-coupled receptor	0.031
46173	H09099	Hs.5378	SPON1	Spondin 1	0.032
258118	N27108	Hs.43886	NA	EST	0.032
260035	N30372	Hs.54434	IRF5	Interferon regulatory factor 5	0.034
324437	W46900	Hs.789	GRO1	GRO1 oncogene	0.040
810859	AA458965	Hs.943	NK4	Natural killer cell transcript 4	0.042
729924	AA399633	Hs.24872	NA	EST	0.045
814478	AA459263	Hs.227817	BCL2A1	BCL2-related protein A1	0.046
712049	AA281635	Hs.315463	IL24	Interleukin 24	0.048
345616	W72431	Hs.82226	GPNMB	Glycoprotein (transmembrane) nmb	0.050
784876	AA448015	Hs.76888	INA	Internexin	0.051
782575	AA447522	Hs.69517	HSJ001348	cDNA for differentially expressed CO16 gene	0.055
767405	AA417921	Hs.85201	CLECSF2	C-type lectin, superfamily member 2	0.056
773106	AA425316	Hs.22142	LOC51700	Cytochrome b5 reductase b5R.2	0.060
181541	H28681	Hs.234074	NA	DKFZp761G02121	0.063
42627	R60995	Hs.21016	COCH	Coagulation factor C homology (cochlin)	0.064
810017	AA455222	Hs.179657	PLAUR	Plasminogen activator, urokinase receptor	0.065
502367	AA134871	Hs.79732	FBLN1	Fibulin 1	0.065
897768	AA598507	Hs.1640	COL7A1	Collagen, type VII, alpha 1	0.070
811740	AA463610	Hs.271986	ITGA2	Integrin, alpha 2 (CD49B)	0.071
285155	N71920	Hs.173560	ODZ2	Odd Oz/ten-m homolog 2	0.075
129112	R10973	Hs.350197	TSG101	Tumor susceptibility gene 101 protein	0.076
191882	H38799	Hs.169764	FLJ20701	Hypothetical protein FLJ20701	0.078
343987	W70234	Hs.44926	DPP4	Dipeptidylpeptidase IV (CD26)	0.078
357278	W93592	Hs.152213	NA	Hypothetical protein FLJ11441	0.079
162208	H25917	Hs.83583	ARPC2	Actin related protein 2/3 complex, subunit 2	0.079
469685	AA027856	Hs.7910	RYBP	RING1 and YY1 binding protein	0.087
31825	R41754	Hs.6496	NA	EST	0.088
291880	N67487	Hs.83551	MFAP2	Microfibrillar-associated protein 2	0.090
280954	N50845	Hs.35089	CNTN3	Contactin 3	0.092
78148	T61649	Hs.177781	NA	Homo sapiens, clone MGC:5618, mRNA	0.094
324901	W49672	Hs.152213	WNT5A	Wingless-type MMTV integration site family	0.095
241066	H91404	NA	NA	EST	0.095
743229	AA400329	Hs.71346	NEF3	Neurofilament 3	0.096
269815	N27159	Hs.727	INHBA	Inhibin, beta A	0.097
321902	W37448	Hs.348710	NA	EST	0.098
485989	AA040170	Hs.251526	SCYA7	Small inducible cytokine A7 (MCP-3)	0.098
46055	H08850	Hs.306480	NA	DKFZp761E2112	0.099
840708	AA488084	Hs.177781	NA	Homo sapiens, clone MGC:5618, mRNA	0.099

Identifiers, names, and functional descriptions are derived from information available in public databases, primarily the National Center for Biotechnology Information (NCBI) UniGene database and GenBank. NA indicates name not available.

*Average value in gene expression of HS-27a (n = 4) was divided by average value in gene expression of HS-5 (n = 4). Sequences that appear twice were specified by 2 different clones on the microarray.

and Cy5, and image-intensity data were extracted and analyzed to obtain expression ratios to Universal RNA for each stromal cell line. From these the expression in the 2 lines could be compared.

Here, we present tables identifying the genes with greater than 10-fold and significant differences (Student *t* test, $P < .05$) in expression between the 2 cell lines (Tables 1 and 2). The interleukin-7 receptor, among the genes with much higher expression in HS-27a, is the subject of a separate manuscript appearing in this issue (Iwata et al, page 1318).[4] A summary table presenting the complete expression profile for each cell line, as well as a comparison between them, for all 17 000 sequences, is available at http://parma.fhcrc.org/MIwata. This site also presents technical details of array spotting, hybridization, cDNA synthesis, and fluor-coupling procedures. The entire raw data set, consisting of 4 microarrays per cell line, is publicly available at http://www.ncbi.nlm.nih.gov/geo/.

Gene expression differences in cytokines/chemokines, G-protein signaling molecules, and multiple extracellular matrix proteins add to the known protein and functional characterization of the lines, leading to new insight into the differences in their support function for hematopoietic progenitors.

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To the editor:

Acidic and neutral sialidase in the erythrocytes of patients with Type 2 diabetes: influence on erythrocyte lifespan

Venerando et al reported an increased quantity of sialic acid at the surface of erythrocytes in diabetic patients and associated the increase with decreased activity of neutral sialidase, an enzyme for which they had previously demonstrated a role in physiologic desialylation of red cells.[1] In their discussion they hypothesized that this excess in sialic acid was responsible for a shorter life span of erythrocytes in diabetes mellitus.

This second assertion is in contradiction with what is commonly known about phagocytosis of senescent red cells. Indeed, several lines of evidence support the contrary hypothesis. The mechanism proposed for this selective recognition and uptake of desialylated red cells is that the macrophage recognizes the adjacent galactose group, which is unmasked by desialylation of glycophorin glycans. Several studies support this hypothesis.

First, in vivo studies showed that neuraminidase-treated erythrocytes are sequestrated more quickly by resident macrophages of the spleen, liver, and bone marrow.[2,3,4] Their life span is also decreased.[2]

Second, centrifugation and lectin recognition studies have showed that older erythrocytes carry less sialic acid residue than younger ones. Moreover, these erythrocytes can be resialylated in vitro, suggesting that the rest of the sialic acid–binding group remains intact. Older red cells can be more resialylated than younger ones.[2]

Third, a receptor for galactose residue has been identified at the surface of peritoneal macrophages that are capable of performing erythrophagocytosis in vitro.[2,3,5]

Fourth, in vitro studies showed that older erythrocytes are preferentially by murine peritoneal macrophages, a reaction that can be inhibited by lactose, which is used as a competitive inhibitor of galactose recognition.[2]

To our knowledge no recent data have invalidated this theory.

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To the editor:

Expression of Ikaros isoforms in patients with acute myeloid leukemia

Recently, Yagi et al[1] reported on expression of Ikaros isoforms in patients with childhood acute myeloid leukemia (AML). Ikaros expression was assessed by nested polymerase chain reaction (PCR) and immunoblotting. The authors found that Ikaros isoform 6 (Ik-6) was detected in 7 of 10 cases of M4 and M5, but in none of the remaining FAB (French-American-British) subtypes. They conclude that the pathogenesis of myelomonocytic/monocytic AML may involve aberrant regulation of apoptosis by Bcl-XL up-regulation due to unscheduled expression of Ik-6.

Over the past several years, there has been a controversy regarding the expression of Ikaros isoforms in human leukemia. Sun et al reported that leukemic cells from infants with B-cell acute lymphoblastic leukemia (ALL) expressed dominant-negative Ikaros isoforms Ik-4, Ik-7, Ik-8, and their deletion mutants.[2] They also reported similar observations with childhood T-cell ALL[3] and childhood ALL[4] using reverse transcriptase (RT) PCR and immunoblotting. Contrary to their reports, we demonstrated overexpression of dominant-negative Ikaros isoform Ik-6 in patients with blast