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THE MARK COVENTRY AWARD

White Blood Cell Gene Expression

A New Approach toward the Study and Diagnosis of Infection

Carl Deirmengian, MD; Jess H. Lonner, MD; and Robert E. Booth, Jr., MD

We introduce a new genomic approach toward the study and diagnosis of infection. Our purpose is to show that synovial fluid white blood cells express a gene expression "signature" that differentiates septic from aseptic inflammation. Synovial fluid was aspirated from patients with acute Staphylococcus aureus infections or acute gout of the knee. Differential cell counts included predominantly neutrophils in all aspirates. Ribonucleic acid was isolated from the synovial-fluid white blood cells and was analyzed on the Affymetrix U133A GeneChip. The neutrophils from a patient whose knee is infected with Staphylococcus aureus can be distinguished from the neutrophils found in gout by nature of their differential gene expression. There are 1615 genes that have an expression level that is significantly different between the groups. The 124 most significant differences are in genes from immune pathways including the interleukin pathway, the tumor necrosis factor pathway, and the antibacterial response. The neutrophils at a site of infection (Staphylococcus aureus) express different genes than the neutrophils at a site of aseptic inflammation (gout). To our knowledge, this is the first in vivo demonstration of this principle. The differences in neutrophil gene expression may be used to develop simple laboratory tests that distinguish the causes of inflammation in a total joint arthroplasty.

Level of Evidence: Diagnostic study, Level II-1. See the Guidelines for Authors for a complete description of levels of evidence.

From 3B Orthopaedics, Pennsylvania Hospital, University of Pennsylvania Health System, Philadelphia, PA.
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Correspondence to: Carl Deirmengian, MD, 3B Orthopaedics, Pennsylvania Hospital, 800 Spruce St., Philadelphia, PA 19107. Phone: 215-720-5551; Fax: 215-829-2253; E-mail: deirmenc@gmail.com.
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The ability to diagnose infection is essential to the care of patients who have had orthopaedic treatment, especially in those who have had a joint arthroplasty.[7,11] Unfortunately, the clinical tests currently used to identify infection often fall short of establishing a firm diagnosis.[1,3,15,16] Pathogen detection techniques such as gram stain and culture often are difficult to interpret. False positive results may be caused by contamination, and false-negative results often are a consequence of presumptive antibiotics.

The host response also is used in judging the probability of infection. For example, in patients who have had a joint arthroplasty, the C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are considered systemic indicators of infection. Additionally, work has been done to quantify the white blood cell (WBC) count in the synovial fluid and tissue in an effort to distinguish infection.[15,16,17,18,19] These methods presume that the magnitude of the host response is associated with the probability of infection but give no specific information regarding the underlying diagnosis.

Although neutrophils are abundant in the acute inflammatory response and almost always are present in an inflammatory joint effusion, relatively little attention has been directed toward their study. Historically, they have been considered uninteresting cells, with a stereotyped inflammatory response that consists of degranulation and phagocytosis. However, as recent genomic studies have changed our understanding of neutrophils, interest in their activity has been reignited.[5,9,21,24,] In vitro, neutrophils have the ability to modulate their genomic responses specifically toward differing causes of inflammation.[24] In fact, they have the ability to respond differently toward varying species of bacteria.[21]

Considering the recent findings related to neutrophil genomic responses, we hypothesized that neutrophils at a site of local inflammation carry information that is diagnostic of the underlying cause of inflammation. With the

TABLE 1. Groups of Patients Involved in Pilot Study: Septic Arthritis versus Gout

Number of Patients	Synovial WBC	Percent PMN	CRP/ESR	Culture	Crystals
S. aureus (N = 7)	90,000 (27–183k)	93%	22/90	Positive S. aureus	None
Gout (N = 5)	10,000 (800–25k)	90%	N/A	N/A	All positive

WBC = White blood cells; PMN = Polymorphonuclear neutrophils; CRP = C-reactive protein; ESR = Erythrocyte sedimentation rate; N/A = Not applicable

recent advances in microarray technology,[6,10,13,22,27,28] which allow for the study of genomic expression patterns, it is possible to compare the gene expression patterns of neutrophils aspirated from the joints of patients with different joint diseases.

Neutrophils never have been shown to exhibit different gene expression profiles in response to different causes of inflammation in vivo. Our purpose is to show that synovial fluid neutrophils have a gene expression "signature" that is specific to the underlying cause of inflammation and that it provides a diagnostic fingerprint discriminating septic from aseptic inflammation.

MATERIALS AND METHODS

This study was approved by the University of Pennsylvania institutional review board, expedited approval. The study groups were chosen with the purpose of comparing septic and aseptic inflammation (Table 1). Acute gout of the knee was chosen as the model for aseptic inflammation because of the ease of establishing a confident diagnosis, without concern of underlying infection. Patients with acute Staphylococcus aureus knee infections were assigned to the infection group. The choice of one bacterial species was made to minimize the theoretical gene expression differences between samples classified as infections.

Over a 15-month period, synovial fluid was collected from the knees of seven patients with S. aureus infections and from five patients with acute gout. All patients with S. aureus infections had fever, acute arthritis, more than one positive S. aureus culture, high CRP values (average, 22; range, 1.9–34), high ESR values (average, 90; range, 39–125), and an elevated synovial WBC count (average, 90,000 cells/mm^3; range, 27,000–183,000; average differential, 93% neutrophils). Five samples were from knees with infected total knee arthroplasty (TKA) prostheses and two samples were from infected natural knees. All patient samples with gout revealed monosodium urate crystals, elevated synovial WBC counts (average, 10,000; range, 800–16,000; average differential, 90% neutrophils), and clinical resolution without antibiotics. All five gout samples were from natural knees.

A new method was developed for the isolation of synovial WBC ribonucleic acid (RNA). Samples of aspirated synovial fluid immediately were added to RNAlater (Ambion Inc., Austin, TX), to prevent RNA degradation and to prevent new transcription. This step assured that RNA profiles did not change after sample collection. Ribonucleic acid was isolated by organic and column techniques and was analyzed by spectrophotometry and the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). All samples had pure, undegraded RNA (Fig 1).

The samples were analyzed on the Affymetrix U133A GeneChip (Affymetrix, Santa Clara, CA) at the core microarray facility at our institution. All protocols were conducted as described in the Affymetrix GeneChip Expression Analysis Technical Manual. Briefly, 5 μg of total RNA was converted to first-strand complementary deoxyribonucleic acid (cDNA) using Superscript II reverse transcriptase primed by a poly(T) oligomer that incorporated the T7 promoter. Second-strand cDNA synthesis was followed by in vitro transcription for linear amplification of each transcript and incorporation of biotinylated cytidine triphosphate (CTP) and uridine 5′ triphosphate (UTP). The cRNA products were fragmented to 200 nucleotides or less, heated for 5 minutes and hybridized for 16 hours to the microarrays. The microarrays then were washed at low [6X SSPE (20× SSPE buffer is 3.0 M NaCl, 0.2 M NaH_2PO_4, 0.02 M ethylenediaminetetraacetic acid pH 7.4)] and high [100 mM MES (2-N-Morpholino-ethanesulfonic acid), 0.1M NaCl] stringency and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. A confocal scanner

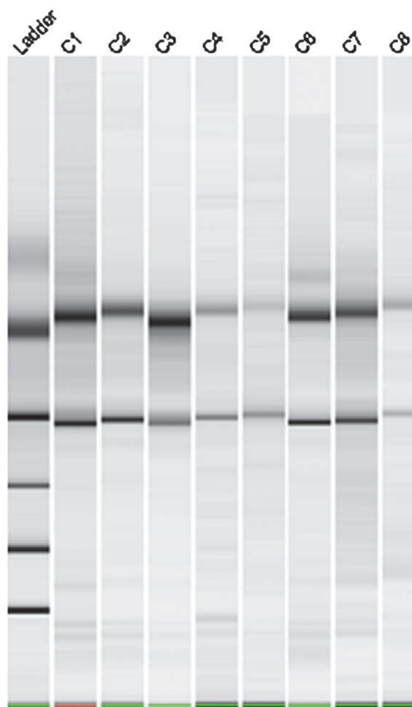

Fig 1. These RNA bands (18s and 28s) reveal intact starting material.

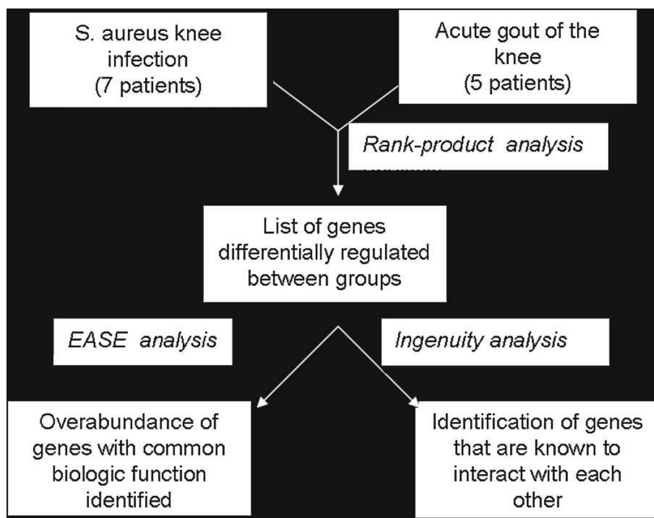

Fig 2. A schematic diagram of data analysis, including the production of a differential gene list by rank product analysis and further validation of expression patterns using expression analysis and Ingenuity Pathway Analysis, is shown.

was used to collect fluorescence signal at 3 μm resolution after excitation at 570 nm. The average signal from two sequential scans was calculated for each microarray feature.

All raw data were sent to the to The Sir Henry Welcome Functional Genomics Facility & Bioinformatics Research Centre at Glasgow University. Rank products and iterative group analysis were done. These are methods that have been shown to be more powerful and reliable when compared to standard methods of detecting differential gene expression.[2,8,12,23]

The list of differential genes identified by rank products analysis (with $p < 0.05$) then was subjected to Expression Analysis Systematic Explorer analysis (EASE), an online microarray analysis provided by the National Institutes of Health (NIH).[4] Expression analysis identifies families of genes based on molecular and biologic function, which are overrepresented in the list of differential genes. Expression analysis with EASE also assigns a p value to the probability of finding an overabundance of genes that are involved in a certain biologic process.

Additionally the list of differentially expressed genes (with $p < 0.05$) was analyzed by pathway analysis (Ingenuity, Mountain View, CA) through the University of Pennsylvania Bioinformatics Core Laboratory. The purpose of pathway analysis is to evaluate the possibility that the genes identified work together and interact through known molecular pathways (Fig 2).

RESULTS

The neutrophils from patients who had acute S. aureus infections in their knees showed a dramatically different gene expression profile when compared with neutrophils from patients who had acute gout. When using the rank-products analysis for the identification of differentially expressed genes, 1615 genes were found to be differentially expressed (each gene with $p < 0.05$) between the groups. One hundred twenty-four of these differentially expressed genes were each associated with a p value between 0.0000001 and 0.0001. The genes found to be differentially expressed in infection relative to gout were evenly divided between up-regulation and down-regulation. The final result of rank products analysis was a list of 1615 genes that were differentially regulated in S. aureus infections versus gout. A heat map of 42 genes that are up-regulated in S. aureus infections is shown in Fig 3.

Patients also were regrouped, in an effort to find differences in gene expression that may be attributed to cell count, the presence of arthroplasty, or antibiotic pretreatment. In this study, the magnitude of the cell count and the presence of arthroplasty were not found to correlate with differences in gene expression. Antibiotic presence before aspirations also did not change the gene expression pattern in this study.

Among the highly up-regulated genes in infection were multiple interleukins, tumor necrosis factors, chemokine ligands, and other genes that previously have been shown to be expressed by in-vitro neutrophils activated by a pathogen[9,24] (Fig 4). Furthermore, among the top 10 up-regulated genes in S. aureus infections were two antimicrobial peptides. Among the genes found to be down-regulated in S. aureus infection compared with those found in gout were tubulins, annexins, and ribosomal proteins, all shown in previous in vitro studies to be down-regulated by pathogen-stimulated neutrophils.[9,24]

The differential gene list produced by rank product analysis then was studied to reveal functional themes. For example, the simultaneous regulation of many genes with similar function provides stronger evidence that a purpose-

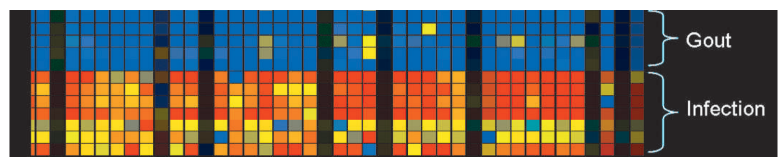

Fig 3. A heat map of gene expression is shown. Each column is a different gene and each row is a different patient. The genes most significantly up-regulated in infection are depicted. Red denotes high gene expression and blue denotes low expression. The gene expression profile clearly differentiates patients with infection versus those with gout. Patients with infection have a gene expression signature that differentiates them from aseptic inflammation.

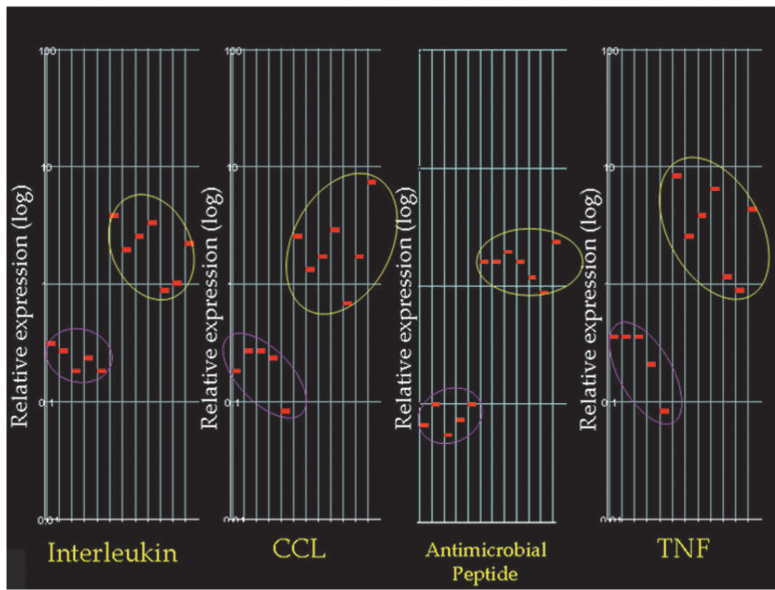

Fig 4. The relative expression of four different genes is graphed. An interleukin family gene, a CCL family gene, an antimicrobial peptide, and a tumor necrosis factor (TNF) family gene are shown. The y-axis is a log scale of gene expression. Each column has data from one patient. For each gene it is evident that patients with infection (yellow circle) have up-regulated the gene expression relative to patients with gout (purple circle). The differences between groups are significant (p = 0.0000001).

ful and concerted change in gene expression was occurring between groups. Additionally, the simultaneous regulation of genes within common pathways would provide insight to the purpose of the change in gene expression.

The list of genes differentially expressed between S. aureus infections and acute gout was analyzed with EASE[4] (Table 2). This analysis tool is used to evaluate the abundance of genes with common biologic purpose within a list of total genes. This is a method of identifying biologic themes within a list of differentially expressed genes. Expression analysis of the 820 genes up-regulated in infection identified enrichment of genes with biologic functions including defense response, immune response, response to pest or pathogen, innate immune response, and apoptosis among others. Twenty-seven percent of the genes that were up-regulated in the infection group are known to have a biologic function in the defense response. The probability that such a high percentage of genes on a list would be part of the defense response, by random chance, is 1×10^{-30}.

The list of genes differentially expressed between infection and gout also was analyzed by Ingenuity software, which identifies genes that are known to interact with each other in biologic pathways. It was found that a number of genes that were up-regulated or down-regulated in infected samples actually interact with each other through known signal transduction pathways. One specific pathway identified involved the proteins signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3) and cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A). Thirty-five genes could be identified that directly or indirectly alter the activity of these proteins (Fig 5).

TABLE 2. EASE Results: Functional Analysis of the Biologic Function of Genes Found to Be Differentially Regulated in Infections versus Gout

Biologic Function	LH	LT	PH	PT	Percent*	Fisher Exact Test (p Value)
Defense Response	107	399	631	7825	27	0.000000000000000000000000000000305
Immune Response	100	399	568	7825	18	0.00000000000000000000000000000019
Response to Pest and/or Pathogen	60	399	371	7825	16	0.000000000000000258
Innate Immune Response	30	399	152	7825	20	0.0000000000929
Apoptosis (Cell Death)	33	399	330	7825	10	0.000144

*Percentage of genes differently expressed between infection and gout that are found to have the given biologic function

LH = Number of genes on the differential list with the given biologic function; LT = Total number of genes on the gene list with any known biologic function; PH = Number of genes on the GeneChip with the given gene function; PT = Total genes on the GeneChip with any known biologic function.

For example, the first row of data shows analysis of genes in the defense response. One-hundred seven of 399 genes on the differential gene list (27%) are involved in the defense response. This is an overabundance compared with the 631 of 7825 genes (8%) on the GeneChip that are known to be involved in the defense response. The p value for this observation is 3×10^{-30}. Therefore, neutrophils from infected knees overexpress defense response genes when compared with neutrophils from knees with acute gout.

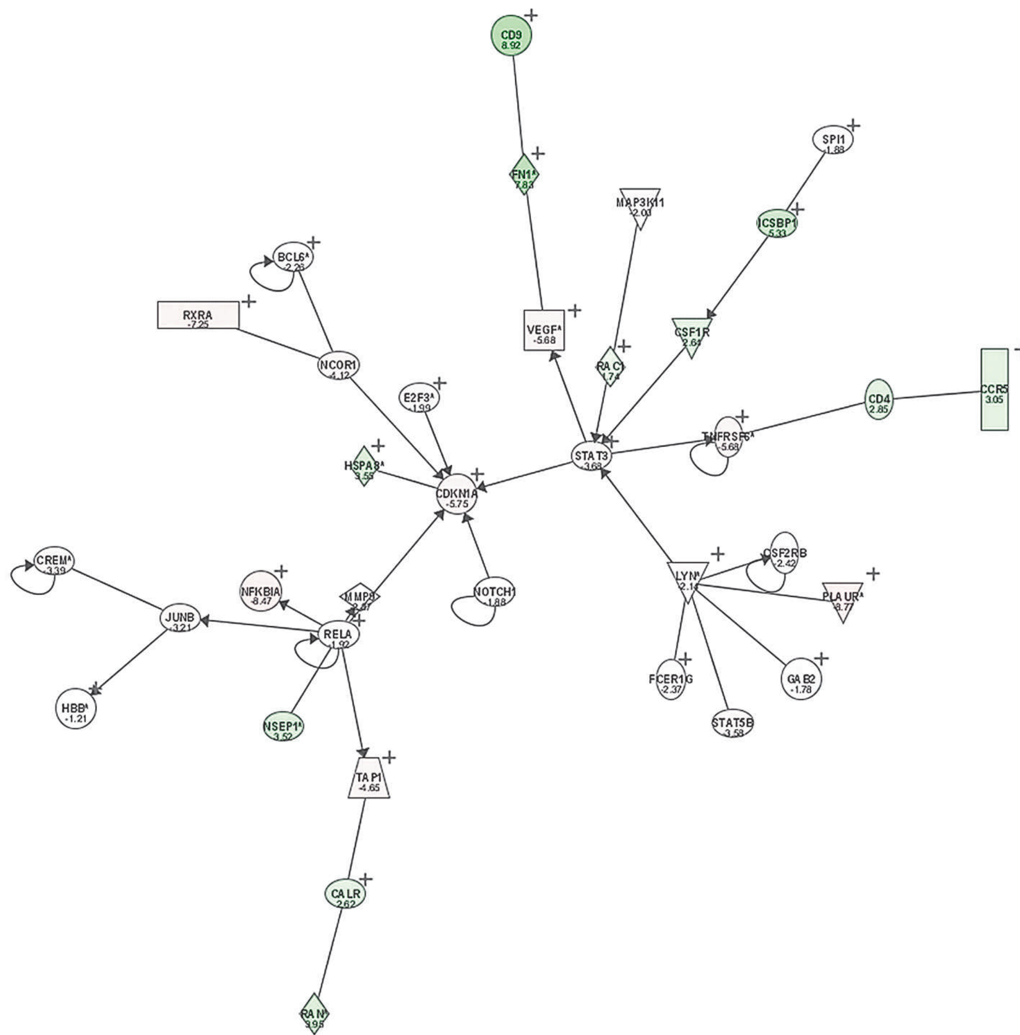

Fig 5. Thirty-five of the genes found to be up-regulated or down-regulated in S. aureus infection versus gout are involved in activating the proteins CDKN1A and STAT3. All of the genes depicted in the pathway in this figure are members of related signal transduction pathways. This concerted regulation of genes in a specific pathway further validates the finding that neutrophils respond to infection with a unique response.

DISCUSSION

The treatment of a painful joint arthroplasty often is associated with ambiguous test results that leave the surgeon without diagnostic confidence.[3,16–18,25] Although systemic indicators of inflammation (CRP, ESR) are valuable, their interpretation may be misleading in postoperative, immunocompromised, or otherwise infected patients. Cultures are a valuable source of information; however indolent bacteria, antibiotic pretreatment, and the formation of glycocalyx all may cause false-negative results. The existing armamentarium of laboratory tests is unable to provide a consistent and accurate diagnosis for the treatment of the painful joint arthroplasty. However, with emerging techniques in molecular biology, a number of strategies may become available for the evaluation of infection.[14,26]

There are potential limitations to this study that can be assessed with further analysis. It could be suggested that the difference in gene expression discovered in this study reflects a preexisting susceptibility, rather than a responsive change. This is highly unlikely considering that the data in this study match in-vitro data from neutrophils stimulated by bacteria. However it is interesting to entertain the idea that inappropriate gene expression results in susceptibility to infection. We currently are investigating whether neutrophils from patients with a history of infected arthroplasty have altered gene expression responses to bacteria.

Another potential limitation is that the patients chosen to be included in the infection group had acute infections with high WBC counts. However, in the clinical setting many joint arthroplasty infections are chronic, with less impressive inflammation. Theoretically, the gene expression signature in these chronic infections should reflect a pattern that is consistent with infection. However, only further investigation, which is currently in progress, will show the gene expression signature of chronic infection.

Another potential limitation of this study is the use of gout as a model for aseptic inflammation. Given the inability to confidently rule out infection in cases of aseptic inflammation, acute gout was chosen as a model. Theoretically, although an aseptic TKA may have a different gene expression signature from gout, the unique pattern of defense gene expression still should differentiate the septic TKA from the aseptic TKA. Only further ongoing studies will show this difference.

Neutrophils historically have been described as uninteresting cells of the innate immune system with a stereotyped activation response consisting of phagocytosis and degranulation. It only has been recently discovered, by in-vitro genomic studies, that neutrophils actually vary their genomic response toward differing causes of inflammation.[9,24] Given this new understanding of neutrophil gene expression, it was hypothesized in this study that examination of the neutrophils in the synovial fluid of an inflamed knee would reveal a specific gene expression signature associated with the etiology of inflammation.

Microarray technology, also known as the GeneChip, provides the ability to study thousands of genes simultaneously, in essence producing a "snapshot" of a cell's genomic state.[6,10,13,22,27,28] In this study, the synovial fluid WBCs (predominantly neutrophils), were collected from the knees of patients with acute S. aureus infections or acute gout. The gene expression in these samples was evaluated with the GeneChip, and it was found that each diagnosis is associated with a unique gene expression signature.

To our knowledge, this is the first in vivo demonstration that neutrophils at the site of local inflammation modulate their pattern of gene expression in specific response to differing etiologies of inflammation. The neutrophils in the synovial fluid of a patient with acute S. aureus infection can be distinguished from those in a patient with acute gout by nature of a unique gene expression signature. There were 1615 genes identified as being different between the groups. There were 124 of these genes associated with p values between 0.0000001 and 0.0001. This represents a dramatic difference between groups. In this study, the difference was not altered by the presence of antibiotics at the time of synovial fluid aspiration.

The list of differentially expressed genes resulting from the analysis was not random, but revealed biologic themes that correspond to the purpose of this study. Among the genes found to be differentially expressed were many, such as interleukin-1, beta (IL1B); chemokine ligands CCL3 and CCL4; and intercellular adhesion molecule 1 (CD54) (ICAM1), which previously have been shown in in vitro studies to be up-regulated in neutrophils exposed to bacteria.[9,24] Furthermore, a significantly high proportion of genes known to be involved in the defense response were found on the list of differentially expressed genes. Some of the genes most up-regulated in the infection group are known to have antimicrobial function. The p value associated with finding such an overabundance of defense genes is 10^{-30}. Therefore, the gene expression signature observed in the neutrophils from infected knees makes biologic sense, reflecting functions that would be expected at a site of infection.

The findings of this study imply a very powerful technique for the diagnosis of infection in joint arthroplasty. Instead of relying on one specific quantitative change, as in many current laboratory tests, a qualitative pattern of change has been shown, which is much easier to identify. In this study, the pattern was independent of antibiotic treatment, avoiding the problem of false-negative results encountered with cultures. It is also highly likely that the local gene expression "signature" exhibited by neutrophils is generated after arrival into the synovial space, circumventing the confusion caused by comorbid diseases such as urinary tract infections and pneumonia. Additionally, if the in-vivo results continue to mirror the in-vitro observations, then neutrophils should also exhibit a unique gene expression signature in response to different species of bacteria.

The GeneChip is currently not reasonable for widespread use as a clinical tool because of high cost. However, the data resulting from GeneChip studies can be used to choose specific markers that are diagnostic for infection. These markers then can be applied in a simpler, cost-effective format that is amenable to widespread clinical use. They could be detected in the synovial fluid by rapid enzyme-linked immunosorbent assay (ELISA) testing in a standard clinical laboratory or tested in the pathology laboratory by histology. Mason et al[17] reported the results of aspirating 86 knees before revision TKA. Neutrophils were abundant in all inflamed synovial fluid, averaging 27% of the WBCs in an aseptic TKA and 73% of the WBCs in a septic knee. Neutrophils are easily accessible for clinical testing.

The treatment of patients with a painful joint arthroplasties would be facilitated by an accurate, consistent test for infection. The differentiation of septic from aseptic inflammation is critical. We have shown that a neutrophil in the synovial fluid of an infected TKA has a unique gene

expression signature that distinguishes it from a neutrophil in an aseptic knee. This represents a powerful new approach toward the diagnosis of infection that circumvents many problems currently associated with blood tests and cultures.

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References

1. Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM: Diagnosis of infection by frozen section during revision arthroplasty. J Bone Joint Surg 77B:28–33, 1995.
2. Breitling R, Armengaud P, Amtmann A, Herzyk P: Rank products: A simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Lett 573:83–92, 2004.
3. Della Valle CJ, Scher DM, Kim YH, et al: The role of intraoperative Gram stain in revision total joint arthroplasty. J Arthroplasty 14:500–504, 1999.
4. Hosack DA, Dennis Jr G, Sherman BT, Lane HC, Lempicki RA: Identifying Biological Themes within Lists of Genes with EASE. Genome Biology. 4:R.70.1–R.70.10, 2003.
5. Fan J, Malik AB: Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. Nat Med 9:315–321, 2003.
6. Frasor J, Danes JM, Komm B, et al: Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: Insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. Endocrinology 144:4562–4574, 2003.
7. Hebert CK, Williams RE, Levy RS, Barrack RL: Cost of treating an infected total knee replacement. Clin Orthop Relat Res 331:140–145, 1996.
8. Hwang D, Schmitt WA, Stephanopoulos G: Determination of minimum sample size and discriminatory expression patterns in microarray data. Bioinformatics 18:1184–1193, 2002.
9. Kobayashi SD, Braughton KR, Whitney AR, et al: From the cover: Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. Proc Natl Acad Sci USA 100:10948–10953, 2003.
10. Kohlmann A, Schoch C, Schnittger S, et al: Molecular characterization of acute leukemias by use of microarray technology. Genes Chromosomes Cancer 37:396–405, 2003.
11. Lavernia CJ, Drakeford MK, Tsao AK, et al: Revision and primary hip and knee arthroplasty: A cost analysis. Clin Orthop Relat Res 311:136–141, 1995.
12. Lee ML, Whitmore GA: Power and sample size for DNA microarray studies. Stat Med 21:3543–3570, 2002.
13. Levene AP, Morgan GJ, Davies FE: The use of genetic microarray analysis to classify and predict prognosis in haematological malignancies. Clin Lab Haematol 25:209–220, 2003.
14. Levine MJ, Mariani BA, Tuan RS, Booth Jr RE: Molecular genetic diagnosis of infected total joint arthroplasty. J Arthroplasty 10:93–94, 1995.
15. Lonner JH, Desai P, DiCesare PE, Steiner G, Zuckerman JD: The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. J Bone Joint Surg 78A:1553–1558, 1996.
16. Lonner JH, Siliski JM, Della Valle C, DiCesare P, Lotke PA: Role of knee aspiration after resection of the infected total knee arthroplasty. Am J Orthop 30:305–309, 2001.
17. Mason JB, Fehring TK, Odum SM, Griffin WL, Nussman DS: The value of white blood cell counts before revision total knee arthroplasty. J Arthroplasty 18:1038–1043, 2003.
18. Musso AD, Mohanty K, Spencer-Jones R: Role of frozen section histology in diagnosis of infection during revision arthroplasty. Postgrad Med J 79:590–593, 2003.
19. Pace TB, Jeray KJ, Latham Jr JT: Synovial tissue examination by frozen section as an indicator of infection in hip and knee arthroplasty in community hospitals. J Arthroplasty 12:64–69, 1997.
20. Pusztai L, Ayers M, Stec J: Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. Clin Cancer Res 9:2406–2415, 2003.
21. Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK: Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: A critical role for monocytes in leukocyte lipopolysaccharide responses. J Immunol 168:4701–4710, 2002.
22. Seth A, Kitching R, Landberg G, et al: Gene expression profiling of ductal carcinomas in situ and invasive breast tumors. Anticancer Res 23:2043–2051, 2003.
23. Simon R, Radmacher MD, Dobbin K: Design of studies using DNA microarrays. Genet Epidemiol 23:21–36, 2002.
24. Subrahmanyam YV, Yamaga S, Prashar Y: RNA expression patterns change dramatically in human neutrophils exposed to bacteria. Blood 97:2457–2468, 2001.
25. Spangehl MJ, Masterson E, Masri BA, O'Connell JX, Duncan CP: The role of intraoperative gram stain in the diagnosis of infection during revision total hip arthroplasty. J Arthroplasty 14:952–956, 1999.
26. Tunney MM, Patrick S, Curran MD, et al: Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 37:3281–3290, 1999.
27. van't Veer LJ: Paik S, Hayes DF: Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530–536, 2002.
28. Zent CS, Zhan F, Schichman SA, et al: The distinct gene expression profiles of chronic lymphocytic leukemia and multiple myeloma suggest different anti-apoptotic mechanisms but predict only some differences in phenotype. Leuk Res 27:765–774, 2003.